OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

Proposition 65

EVIDENCE ON THE DEVELOPMENTAL TOXICITY OF

Cannabis (Marijuana) Smoke and Δ^9-THC

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Reproductive and Cancer Hazard Assessment Branch
Office of Environmental Health Hazard Assessment
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Preface

Proposition 65[1] requires the publication of a list of chemicals "known to the state" to cause cancer or reproductive toxicity. The Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency maintains this list in its role as lead agency for implementing Proposition 65. The Developmental and Reproductive Toxicant Identification Committee (DARTIC) advises and assists OEHHA, and serves as the state's qualified experts for determining whether a chemical has been clearly shown to cause reproductive toxicity[2].

As of January 1, 2018, the adult use of cannabis is legal under California law (Medicinal and Adult-Use Cannabis Regulation and Safety Act[3]). Cannabis use during pregnancy has been reported, and legalization may increase such use. In light of possible public health concerns related to use during pregnancy, the Director of OEHHA, in consultation with the Chair of the DARTIC, determined that cannabis and cannabis-related chemicals should be reviewed for consideration for listing under Proposition 65 as causing reproductive toxicity (developmental endpoint).[4]

On March 15, 2019, OEHHA issued a public request for information on the developmental toxicity of cannabis (marijuana), marijuana (cannabis) smoke, cannabis extracts, and delta-9-tetrahydrocannabinol (Δ^9-THC). Nine submissions were received and considered during the development of this document. Because of the large volume of data available, OEHHA has limited the review of the evidence to the developmental toxicity of cannabis smoke and Δ^9-THC. Other extracts of cannabis may be considered at a later time.

On December 11, 2019, the DARTIC is scheduled to deliberate on the reproductive toxicity (developmental endpoint) of cannabis (marijuana) smoke and Δ^9-THC. OEHHA developed this document as part of the hazard identification materials that are provided to the DARTIC to assist it in its deliberations on whether or not cannabis (marijuana) smoke, or Δ^9-THC, or both, should be listed under Proposition 65. To the extent possible, the original papers discussed in the document are provided to the DARTIC.

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[1] The Safe Drinking Water and Toxic Enforcement Act of 1986 (codified at California Health and Safety Code section 5249.5 *et seq*.).

[2] Title 27, Cal. Code of Regs., section 25305(b)(1)

[3] The Medicinal and Adult-Use Cannabis Regulation and Safety Act is available at: https://leginfo.legislature.ca.gov/faces/codes_displayexpandedbranch.xhtml?tocCode=BPC&division=10.&title=&part=&chapter=&article

[4] In accordance with "Process for Prioritizing Chemicals for Consideration under Proposition 65 by the 'State's Qualified Experts" Available at https://oehha.ca.gov/media/downloads/proposition-65/document/finalpriordoc.pdf

OEHHA is holding a public comment period on this hazard identification document. Comments on this document will be included in the hazard identification materials that are provided to the DARTIC members prior to the meeting.

Contents

Acronyms and Abbreviations

Δ^8-THC	Delta-8-tetrahydrocannabinol
Δ^9-THC	Delta-9-tetrahydrocannabinol
µg	Microgram
11-OH-THC	11-hydroxy -Δ^9-THC
THC-COOH	Carboxy -Δ^9-THC
2-AG	2-arachidonylglycerol
2meH3K9	Dimethylated lysine 9
3meH3K	Trimethylated lysine 4
AA	Absolute alcohol
AC	Adenylyl cyclase
ADHD	Attention deficit disorder with hyperactivity
AEA	N-arachidonylethanolamide (anandamide)
Ahr	Aryl hydrocarbon receptor
AHRR	Aryl hydrocarbon receptor repressor
AJA; CT-3; IP-751	Ajulemic acid
Akt/PKB	Protein kinase B
anandamide, AEA	N-arachidonylethanolamide
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
AOR	Adjusted odds ratio
assoc	Association
avg	Average
BD	Birth defects
BL	Birth length
BNBAS	Brazelton Neonatal Behavioral Assessment Scale
BSID	Bailey Scales of Infant Development
btwn	Between
BW	Birth weight
Ca2+	Calcium
cAMP	Cyclic adenosin monophosphate
CB_1R	Cannabinoid receptor 1
CB_2R	Cannabinoid receptor 2
CB_3R (GPR55)	Cannabinoid receptor 3
CBD	Cannabidiol
CBN	Cannabinol
CBR	Cannabinoid receptor
CBV	Cannabivarol
Chr	Chromosome
Cnb	Cannabis
CNS	Central nervous system
CO	Carbon monoxide
conc	Concentration
COR	Crude odds ratio
COX	Cyclooxygenase
CP55,940	CBR agonist

CRH	Corticotropin releasing hormone
d	Day
DAVID	Database for Annotation, Visualization and Integrated Discovery
DES	Diethylstilbestrol
DFA	Discriminant Function Analysis
diff	Difference
dist	Distribution
DMH-CBD	(-)-5'-(1,1-dimethylheptyl cannabidiol)
DMRs	Differentially methylated regions
DNMT1	DNA methyltransferase enzyme 1
DNMT3a	DNA methyltransferase enzyme 3a
DOB	Date of birth
Drd 2	Dopamine receptor D2
Drd1	Dopamine receptor D1
EC	Endocannabinoid (system)
eCB(s)	Endogenous cannabinoid (s)
eCBs	Endogenous cannabinoids
ELISA	Enzyme-linked immunosorbent assay
EPSP	Excitatory post synaptic potential
exp	Exposure
F0	Parental generation
F1	All offspring from the parent (F0) generation
FAAH	Fatty acid amide hydrolase
FAS	Fetal alcohol syndrome
FGR	Fetal growth restriction
fMRI	Functional magnetic resonance imaging
freq	Frequency
g	Gram
GA	Gestational age
GC-MS	Gas chromatography-mass spectrometry
GD	Gestational day
GDM	Gestational diabetes mellitus
GDP	Guanosine diphosphate
GHT	Gestatational hypertension
GluRs	Glutamate receptors
GO	Gene ontology
GPCR	G-protein coupled receptor
GTP	Guanosine triphosphate
HC	Head circumference
HOME	Home Observation for Measurement of the Environment
HPA	Hypothalamic–pituitary–adrenal [axis]
IBR	Infant Behavior Record (from BSID)
ICM	Inner cell mass
IL-6	Interleukin-6
incl	Included
info	Information
IPSP	Inhibitory post synaptic potential
IQR	Interquartile range

IUGR	Intrauterine growth restriction/restricted
JAKs-STAT	Janus kinase signal transducers
JNK	C-Jun N-terminal kinase
LBW	Low birth weight
LGA	Large for gestation age
LIF	Leukemia inhibitory factor
LMP	Last menstrual period
LOD	Limit of detection
LOEL	Lowest observed effect level
LOQ	Limit of quantification
LOX	Lipoxygenase
LTD	Long term potentiation
LTD	Long term depression
MAGL	Monoacyl-glycerol lipase
MDI	Mental Developmental Index (from BSID)
mg	Milligram
ml	Milliliter
mo / mos	Month / months
N	Population size
n	Sample size
NAc	Nucleus accumbens
NAPE-PLD	N-acylphosphatidylethanolamine-hydrolyzing PLD
NBAS	[Brazelton] Neonatal Behavioral Assessment Scale
NE	Norepinephrine
NICHD	National Institute of Child Health and Human Development
NICU	Neonatal intensive care unit
NIDA	National Institute on Drug Abuse
NMDA	N-methyl-D-aspartate [receptor]
NO	Nitric oxide
ns	Not statistically significant
NSDUH	US National Survey on Drug Use and Health
NTD	Neural tube defect
OR	Odds ratio
oz	Ounce(s)
PA	Placental abruption
PAHs	Polycyclic aromatic hydrocarbons
PDI	Psychomotor Developmental Index (from BSID)
PFC	Prefrontal cortex
PI	Ponderal index
PLC	Phospholipase
PND	Postnatal day
PPROM	Preterm Premature rupture of membranes
preg	Pregnancy
PROM	Premature rupture of membranes
PTB	Preterm birth
PTD	Preterm delivery
PVN	Paraventricular nucleus
RhoA	Ras homolog gene family, member A

RR	Relative risk
SAB	Spontaneous abortion
SB	Still birth
sc	Subcutaneous
SD	Standard deviation
SE	Standard error
SEM	Standard error of the mean or median
SES	Socioeconomic status
SGA	Small for gestational age, also referred to as intrauterine growth restriction (IGR, IUGR), fetal growth restriction (FGR)
SIDS	Sudden infant death syndrome
ss	Statistically significant
STAT3	Signal transducer and activator of transcription 3
STD	Sexually transmitted diseases
STP	Short term potentiation
suppl	Supplement
TE	Trophectoderm
THCA	Tetrahydrocannabinolic acid
TSS	Transcription start site
U/S	Ultrasound
VEH	Vehicle
Virodhamine	O- arachidonoyl ethanolamine
VLBW	Very low birth weight
VSD	Ventral Septal Defect
w/	With
w/in	Within
w/o	Without
WIN	WIN 55212-2, an aminoalkylindole, CBR agonist
WISC	Wechsler Intelligence Scale for Children
wk	Week
yo	Years old
yr / yrs	Year / years
α1-AR	α1-adrenoceptors
β2-AR	β2-adrenoceptors

Executive Summary

Cannabis has been used for medicinal and psychotropic purposes in some parts of the world for thousands of years. Inhaling cannabis smoke for its psychotropic properties became popular in western cultures in the twentieth century. Cannabis smoke is formed when the dried flowers, leaves, stems, seeds and resins of plants in the genus *Cannabis* are burned. Cannabis smoke aerosol contains thousands of organic and inorganic chemicals, including psychoactive cannabinoids, which are unique to *Cannabis* plants. Δ^9-THC (delta-9-tetrahydrocannabinol) is understood to be the most potent psychoactive compound present in cannabis.

Available Data

This document compiles and summarizes studies on the developmental effects of cannabis smoke and Δ^9-THC. Numerous epidemiology studies have explored the potential for prenatal and early-life exposure to cannabis smoke and constituents of cannabis to affect development. A range of experimental studies, mostly in rodents, have also investigated the potential to cause developmental harm. Some of the studies use emerging techniques evaluating effects at the cellular and molecular level. This document compiles, organizes and summarizes this large body of evidence. It focuses on cannabis smoke and Δ^9-THC and evidence that is directly or indirectly relevant to determining whether these substances can cause developmental toxicity. This document aims to present the body of data to support an objective and full consideration of the evidence.

Δ^9-THC is one of at least 60 different "exogenous" cannabinoid compounds contained in *Cannabis* plants. Our bodies naturally produce cannabinoids as part of the endocannabinoid (EC) system. This system has many physiological roles, including supporting pregnancy, reproductive function, bone growth, regulation of the immune system, and neurodevelopment. The EC system is important during early brain development, which has prompted considerable research. However, current understanding of the mechanisms through which neurodevelopment and neurobehavior are shaped by the EC system remains limited. Still, mechanistic evidence presented here from various lines of inquiry, including epigenetic studies, provide insight as to how Δ^9-THC may play a role in causing significant biological outcomes. Mechanistic studies of various types (in humans and experimental animals, both *in vivo* and *in vitro*) are presented that investigate the potential for maternal cannabis exposure to alter stress hormone status and/or the expression of specific genes and/or proteins thought to underlie behavior and other neurological changes associated with effects in offspring potentially occurring soon or long after exposure has ended.

Somatic Developmental Effects

There are numerous human and animal studies evaluating the potential for cannabis smoke, Δ^9-THC and cannabis extracts to cause somatic developmental effects from mothers' cannabis use during pregnancy. This includes effects on embryo development and implantation, the fetus, and a number of birth outcomes. Human, non-human primate, rodent, rabbit and zebrafish studies are presented.

Human Studies

Somatic developmental outcomes evaluated in human studies include postnatal growth, pre-, peri-, and postnatal mortality, birth defects, body weight, gestational age, and preterm birth.

Postnatal Growth

Seven epidemiologic studies evaluated the effects of prenatal cannabis exposure on postnatal growth. All seven were prospective cohort studies and examined the association of cannabis exposure throughout pregnancy and postnatal growth. Growth measures included height, weight, head circumference, body mass index (BMI), ponderal index (PI), skinfold thickness, and weight-for-height *Z* score. Age at follow-up ranged from one to sixteen years. All studies based exposure on self-reporting.

Heavy prenatal cannabis use was associated with heavier and taller children at 24 months (Fried and O'Connell 1987), reduced head circumference at 9-12 years (Fried, Watkinson et al. 1999), and a non-significant reduction in head circumference (Fried et al. 2001) at 13-16 years; differences in body size did not persist. The cannabis users in this study consumed more calories and protein than non-users in this predominantly low-risk, middle socioeconomic status (SES) cohort (Fried et al. 1999). Two of three studies from a high-risk, low SES cohort of teenage mothers reported no differences in weight, height, and head circumference at three and six years (Day et al. 1992, Day et al. 1994a). The third study reported no differences in weight, head circumference, adiposity, or proportionality, but did reporting significantly shorter stature for six-year-old children with prenatal 2nd trimester cannabis exposure (Cornelius et al. 2002).

Pre-, Peri-, Postnatal Mortality

Eleven studies examined associations between prenatal cannabis use and spontaneous abortion (SAB), stillbirth (SB), perinatal mortality, and sudden infant death syndrome (SIDS). Most of these studies were not focused on fetal viability or perinatal mortality as a primary outcome; many had few cases. Therefore, while some studies, including a meta-analysis, did report associations with SAB, SB, or perinatal mortality, they were unable to report results adjusted for covariates (Varner et al. 2014, Conner et al. 2016, Coleman-Cowger et al. 2018, Howard et al. 2019). In one very large retrospective cohort study, the risk of SB was significantly greater among women diagnosed with

"cannabis abuse" or "cannabis dependence", OR=1.50 (1.39, 1.62), p<0.0001 (Petrangelo et al. 2018). Of two case-control studies examining prenatal cannabis exposure as a risk factor for SIDS, neither reported significant associations with maternal prenatal cannabis use (Scragg et al. 2001). Another case-control study reported that SIDS was associated with paternal cannabis use during the conception period, OR=2.2 (1.2, 4.2), and pregnancy, OR=2.0 (1.0, 4.1), adjusted for paternal postnatal tobacco smoking and alcohol use during conception.

Birth Defects

Thirteen epidemiologic studies evaluated associations between prenatal cannabis exposure and birth defects, including various minor and major malformations, ventricular septal defects (VSD), fetal alcohol syndrome (FAS)-like features, or unspecified birth defects. Associations with VSD were observed for maternal exposure (Williams et al. 2004) and paternal exposure (Ewing et al. 1997). Other investigators reported associations between prenatal cannabis use and anencephaly (Van Gelder et al. 2009), and FAS-like features (Hingson et al. 1982). Birth defects were often one of many types of birth outcomes examined in a given study. Studies that reported statistically significant associations between cannabis and birth defects typically focused on birth defects rather than multiple types of birth outcomes, and assessed exposure during early pregnancy (Williams et al. 2004, van Gelder et al. 2009) and spermatogenesis (Ewing et al. 1997).

Birthweight

Twenty-seven studies examined the association between birthweight (BW) and prenatal cannabis exposure, of which 12 reported associations with prenatal cannabis use, adjusted for prenatal tobacco use. The remaining 13 studies found no significant associations for cannabis adjusted for tobacco. Two studies suggested a dose-response relationship between prenatal cannabis exposure and lower birthweight (Hingson et al. 1982, Saurel-Cubizolles et al. 2014). Of the 10 studies published in the past decade, seven reported significantly lower birthweight in infants prenatally exposed to cannabis, adjusted for prenatal tobacco exposure.

Gestational Age

Eighteen studies examined the association between prenatal cannabis exposure and gestational age (GA), of which 12 did not report significant associations between GA and prenatal cannabis use. Four studies reported that cannabis use was associated with shorter gestation or lower GA (Fried et al. 1984, Cornelius et al. 1995, Saurel-Cubizolles et al. 2014, Howard et al. 2019). Three studies reported a reduction of gestation by roughly one week associated with prenatal cannabis use (Fried et al. 1984, Cornelius et al. 1995, Howard et al. 2019). Fried et al. (1984) also reported a dose-dependent relationship of decreasing GA with increasing cannabis exposure among

heavy users. One study reported a positive association between total number of joints smoked in pregnancy and GA (Tennes et al. 1985).

Preterm Birth

Nineteen studies examined the association between prenatal cannabis exposure and preterm birth (PTB). Six studies reported statistically significant associations with PTB. Four of the six studies that reported associations with PTB analyzed more than one level of prenatal cannabis exposure, rather than comparing any cannabis use with no cannabis use (Gibson et al. 1983, Saurel-Cubizolles et al. 2014, Conner et al. 2016, Leemaqz et al. 2016) and were therefore able to distinguish light or occasional use from heavier use. Additionally, although Petrangelo et al. (2018) did not quantify cannabis exposure, the exposure definition likely included mainly very heavy cannabis use. Two studies reported borderline significant associations between prenatal cannabis exposure and PTB (Hatch and Bracken 1986, Shiono et al. 1995), and 13 studies did not report significant associations with PTB.

Animal Studies

Animal studies were conducted using *in vivo*, *ex vivo* or *in vitro* model systems, to evaluate effects on embryo development and implantation, and later stages of embryo and fetal development in various species.

Implantation

Embryonic development and successful implantation requires coordination between components of the EC system in the oviduct and endometrium with those in the embryo. In the mouse, cannabinoid receptor 1 (CB_1R) and cannabinoid receptor 2 (CB_2R) are expressed in early embryos (Paria et al. 1995), fetal membranes, the reproductive tract, and the placenta (Taylor et al., 2007). CB_1R messenger RNA (mRNA) has been detected in preimplantation 4-cell embryos through the blastocyst stage, and CB_2R mRNA has been detected in mouse 1-cell embryos through the blastocyst stage (Paria et al. 1995). In vitro Δ^9-THC exposure of 2-cell mouse embryos inhibited development to the blastocyst stage (Paria et al. 1995). In vivo studies reported that prenatal Δ^9-THC exposure had no effect on mouse embryo implantation (Paria et al. 1992) unless mice Δ^9-THC metabolism was inhibited (Paria et al. 1998). Under conditions where Δ^9-THC metabolism was inhibited, significantly lower rates of embryo (blastocyst) implantation were reported, compared to controls (Paria et al. 1998; Paria et al. 2001).

Growth

In rodent studies of prenatal cannabis smoke exposure, several investigators have reported significant effects on pre- and postnatal growth. Delays in acquisition of postnatal developmental landmarks were also reported. However, all of the studies reporting statistically significant effects performed their analyses on a per group, not a

per litter basis. Where analysis was presented on a per litter basis, statistical significance was not achieved. In addition, many of the studies are limited by inadequate reporting, poor experimental methodology, and inadequate or marginally adequate group size.

EC system signaling is involved in bone growth and remodeling at all stages of life, as indicated in studies of CBR knock out mice. Studies in cell culture have explored the actions of exogenous cannabinoids, including Δ^9-THC, to affect bone growth. For example, in vitro exposure of mouse primary chondrocyte cultures to Δ^9-THC was reported to inhibit chondrocyte differentiation (Wasserman et al. 2015), while daily administration of Δ^9-THC to mice from five to 11 weeks of age decreased femoral length in female pups (Wasserman et al. 2015). Studies of bone growth and prenatal exposure to Δ^9-THC in animals are not available.

Viability and Mortality

In rodent and rabbit studies of pre-conceptional or prenatal oral exposure to Δ^9-THC, several studies reported general fetal and pup toxicity, including increased fetal, perinatal, or postnatal offspring mortality in eight studies; decreased fetal or birth weights in seven studies; and altered hormone levels or fertility in F1 male offspring in six studies. In one of the larger prenatal exposure studies in rodents employing a control and three dose groups, with 50 pregnant female rats/dose group, a statistically significant decrease in the number of viable fetuses per litter and a statistically significant increase in whole litter resorptions was observed in all dose groups Fleishman et al. (1980). In a similarly designed study of prenatal exposure to Δ^9-THC in mice, these same authors reported decreases in fetal viability and increases in whole litter resorptions in all dose groups. Several of these oral route studies in rodents and rabbits, as well as the single study conducted in chimpanzees, are limited by inadequate reporting of methods and results.

In rodent and rabbit studies of pre-conceptional or prenatal exposure to Δ^9-THC exposure by parenteral routes, a number of studies reported decreases in fetal or birth weights, and increases in fetal, perinatal, or postnatal offspring mortality. Most of these studies are limited by inadequate reporting, marginally adequate group size, and lack of statistical analysis on a per litter basis. The study conducted in Rhesus monkeys reported an apparent effect of Δ^9-THC daily intramuscular injection, starting around GD 21-35, and continuing throughout gestation, with four of the five pregnancies lost in Δ^9-THC exposed females (three early spontaneous abortions and one stillbirth), compared to none lost among the five controls. Information on the reproductive histories of the individual females used in the studies was not provided, nor were colony statistics for pregnancy loss versus live births.

Immune System

The effects of Δ^9-THC on the developing immune system have been studied in a series of experiments in mice by Lombard et al. (2011). Fetal and adult thymocytes show similar patterns of CB_1R and CB_2R mRNA expression, and prenatal exposure (gestation day 16) was associated, in a dose-dependent manner, with a decrease on the following day in mouse fetal thymic cellularity and a corresponding increase in T cell apoptosis. Decreases were observed in several T cell subpopulations. Pretreatment of the dams with antagonists of either CB_1R or CB_2R prior to Δ^9-THC administration ameliorated these effects. Additional studies reported that the decreased thymic cellularity and increased apoptosis resulting from prenatal Δ^9-THC exposure lasted for several days and was present the day after birth, and one week after birth. Other studies are consistent with these observations, and in addition have reported decreased T cell function in prenatally exposed mice one week after birth.

Neurodevelopmental Effects

There are numerous human and animal studies evaluating the potential for cannabis to cause neurodevelopmental effects from mothers' cannabis use during pregnancy. This includes effects on motor behavior, cognitive performance and emotionality, including effects expressed at later life stages. Studies of the effect of Δ^9-THC exposure on the susceptibility to discriminative and reinforcing effects of drugs of abuse later in life were also summarized. In addition to human, primate and rodent studies, data from Zebrafish are presented.

Locomotor Activity

Epidemiological studies and controlled animal investigations have explored the potential for cannabis or Δ^9-THC to affect locomotor activity. Some animal studies reported no change in locomotor activity (Vardaris et al. 1976, Brake et al.1987, Navarro et al.1996,Trezza et al. 2008) while other studies, after maternal exposure to cannabis during pregnancy, reported temporary changes in locomotor activity in offspring (young and adult) (Fried 1976; Navarro et al. 1995; Trezza et al. 2008; Vardaris et al. 1976). A further study reported that perinatal exposure to Δ^9-THC resulted in less activity in the open field tests when male, but not female, offspring were pretreated with dopamine agonists (Moreno et al. 2003). Still another study reported parental Δ^9-THC exposure resulted in decreased movements in female, but not male, offspring (Szutorisz et al. 2016). Finally, adult offspring of Δ^9-THC-exposed males were reported to have significantly more rapid habituation of locomotor activity, with the female offspring being more active than the male offspring (Levin et al. 2019).

The mechanistic studies related to the potential for cannabis to cause motor dysfunction covered multiple possible pathways. During neurodevelopment, in the central nervous system (CNS), there is a switch from CB_2R to CB_1R expression where CB_1Rs become

upregulated in postmitotic neurons and CB_1R expression predominates (Maccarrone et al. 2014). Activation of CB_1R evokes transient Ca^{2+} elevation through various pathways, including phospholipase C, and voltage dependent and other calcium channels. Calcium homeostasis is crucial in synaptic plasticity, proper neuronal development, and skeletal muscle function. In *in vitro* studies, cannabinoids were reported to affect various targets that alter Ca^{2+} homeostasis in both the peripheral and central nervous systems (Ahmed et al. 2016; Antonelli et al. 2004; Gkoumassi et al. 2009; Newman et al. 2007; Olah et al. 2016; Lauckner et al. 2005; Rao and Kaminski 2006; Zhuang et al. 2004). In zebrafish embryos, exposure to Δ^9-THC during the gastrulation period of development was reported in one recent study to affect motor neuron morphology changes, synaptic activity at the neuromuscular junction (NMJ), and locomotor responses to sound (Ahmed et al. 2018).

Cognitive Function

Epidemiological studies and controlled animal investigations have explored the potential for cannabis or Δ^9-THC to affect cognition. Several rodent studies explored the potential effects of prenatal exposure to cannabis extracts or Δ^9-THC on cognitive function, many without effect and a number reporting changes in different aspects of cognitive function. In one early study, offspring prenatally exposed to cannabis extract were reported to commit significantly more errors in maze activities and to spend significantly more time in the maze (Gianutsos and Abbatiello, 1972). In another study, perinatal Δ^9-THC exposure was associated with long-term memory impairment and disruption in short-term olfactory memory in adult offspring. These cognitive impairments were associated with alterations in the cortical expression of genes related to glutamatergic neurotransmission, together with a decrease in adult cortical extracellular levels of glutamate (Campolongo et al. 2007). Perinatal exposure of rats to Δ^9-THC was also associated with deficits in learning in adulthood (O'Shea and Mallet 2005). The exposed rats were observed to commit significantly more errors, and had working memory deficits in the delayed alternation task. In a recent study with Δ^9-THC, long-lasting significant impairment in attentional performance as well as a significant increase in habituation of locomotor activity was reported in paternally exposed offspring that were tested during adulthood (Levin et al., 2019). This study included tests evaluating attention and various aspects of memory impairment.

A number of human studies evaluated the effects of prenatal cannabis exposure on various aspects of offspring's cognitive function. The studies spanned from early childhood to adolescence and specifically explored outcomes relating to visual function and processing, attention, and intelligence and academic achievement. In addition, a number of studies evaluated the effects of prenatal cannabis exposure on maturation of the central nervous system.

Visual Function and Processing

In studies evaluating visual function and processing, three report effects on higher order cognitive processes related to visual analysis in exposed offspring, with no effects on basic visual functions (Chakraborty et al. 2015, Fried et al. 1998, Fried and Watkinson 2000). Specifically, in Fried and Watkinson (2000) exposed offspring had lower subtest scores related to planning, integration, analysis, and synthesis. Likewise, Fried et al. (1998) found lower Block Design task scores and Picture Completion test scores in exposed offspring compared to unexposed ones. The tests used were designed to assess perceptual organization, spatial visualization or abstract conceptualization, and an ability to differentiate essential from nonessential details. A neuroimaging study reported a significant reduction in the right precentral gyrus/premotor cortex – the region of the brain that reflects the encoding process in visuospatial short-term memory – in exposed compared to unexposed offspring.

Attention

Several epidemiologic studies assessed the relationship between prenatal cannabis exposure and attention. Studies evaluated attentional behavior from early childhood to adolescence. Many studies reported increased impulsivity in offspring who had been prenatally exposed to cannabis, specifically in performance on Continuous Performance tests (Fried et al. 1992a, Leech et al. 1999, Fried et al. 1988, Richardson et al. 2002). Several studies also reported associations between prenatal cannabis exposure and deficits in sustained attention in offspring through varying methods (Fried et al. 1992a, Fried and Watkinson 2001, Goldschmidt et al. 2000, Goldschmidt et al. 2012). In some studies, these attention problems were also linked to other outcomes later in life such as delinquency and lower school achievement (Goldschmidt et al. 2000 and Goldschmidt et al. 2012). A number of studies found no association between some aspect of attentional behavior and prenatal cannabis use, and one study (Leech et al. 1999) found improved attentional behavior (O'Connell and Fried 1991, Fried et al. 1998, Fried and Watkinson 2001, Richardson 2012, Noland 2005, and Rose Jacobs et al. 2017).

Intelligence

Several studies evaluated the association between prenatal cannabis use and intelligence and academic achievement. These studies used a wide battery of tests and varying methods to assess different aspects of intelligence, such as reading, language development, IQ, verbal skills, quantitative skills and more. These studies also covered children of different ages and environments. This complex dataset is discussed in section D.

Central Nervous System Maturation:

Multiple studies used the maturation of visual pathways as a mechanism for assessing the maturation of the central nervous system (Tansley 1987, Scher 1998). Habituation

to light in infancy and sleep arousals are also considered indicators of CNS maturity (Fried and Makin 1987 and Scher et al. 1988). A majority of studies investigating maturation of the central nervous system reported associations between prenatal exposure to cannabis and indicators of altered CNS maturation. These included statistically significant results for: responsiveness and habituation to visual stimuli (Fried 1980, Fried and Makin 1987) during the first month after birth, but not at older ages (Fried 1982); visual evoked potentials (Tansley et al. 1987, Scher et al. 1998); and sleep (Scher et al. 1988, Dahl et al. 1995).

Emotionality

Studies indicate endocannabinoids play a role in modulating emotionality (Campolongo et al. 2009; Trezza et al. 2008). Epidemiological studies have explored the potential for cannabis or Δ^9-THC to affect emotionality. In addition, several mammalian studies have explored the potential for emotionality in offspring after perinatal exposure (in utero and lactational) to Δ^9-THC:

- emotional reactivity in rats was reported (Navarro et al. 1994).
- in a rodent model designed to study anxiety, a dose-dependent and environment-dependent anxiolytic and/or anxiogenic effect was reported, including an increased rate of separation-induced ultrasonic vocalizations, reduced social interaction and play behavior, and increased generalized anxiety behavior (Trezza et al., 2008).
- increased social interaction in males and decreased time in the inner part of the open field was reported (Newsom and Kelly 2008).

Drug Sensitivity Later in Life

Epidemiological studies and controlled animal investigations have explored the potential for cannabis or Δ^9-THC to affect drug and alcohol use behaviors, and susceptibility to addiction. Several animal studies examined the potential for enhanced adult experience or increased frequency of use of alcohol or opiates after pre- or perinatal exposure to Δ^9-THC. Following Δ^9-THC exposure in utero or via lactation, adult animals were reported to have an increased rate of acquisition of morphine self-administration and or enhanced sensitivity towards the rewarding effects of morphine or heroin, such as spending more time in a morphine-paired compartment than in a saline-paired compartment (Navarro et al. 1994, Navarro et al. 1995, Navarro et al. 1996, Rubio et al. 1995, Rubio et al. 1998, Vela et al. 1998, Singh et al. 2006). One study reported an increase in heroin-seeking behavior in adult males, but only during mild stress and drug extinction (Spano et al. 2007). Other studies reported no effects on food or morphine self-administration (Gonzalez et al. 2003) or ethanol self-administration (Economidou et al. 2007) following perinatal exposures.

Because of the length of time between prenatal exposure and outcomes, and concomitant exposures, epidemiological studies are challenging. In human fetuses collected after elective abortions, maternal cannabis use was associated with decreased *DRD2* mRNA levels in the nucleus accumbens (NAc) (DiNieri et al., 2011). The NAc is associated with a number of behaviors, including compulsive behaviors, addiction vulnerability, and reward sensitivity. Similarly, after perinatal exposure to Δ^9-THC, altered mRNA levels first in the NAc and later in the dorsal striatum were observed in rats (Szutorisz et al. 2014). Also in the offspring of adolescent female rats exposed to Δ^9-THC, an anhedonic phenotype with lower sensitivity to natural rewards and susceptibility to addictive behaviors was reported (Pitsilis al., 2017).

Other Neurodevelopmental Effects

No effect on auditory startle was reported at on postnatal days 57–60 in both male and female rats exposed during gestation (Hutchings et al. 1991). An increase in the frequency and time spent grooming in both adult males and females after oral perinatal exposure to Δ^9-THC was observed (Navarro et al. 1995). In addition, some researchers (Dalterio et al, 1984; Fried and Charlebois 1979; Navarro et al. 1996) have reported on studies indicating that maternal exposure to cannabis or cannabis extracts also altered the pattern of approach to sexually receptive females by male rat offspring.

A variety of additional neurodevelopmental outcomes reported in human studies are presented in Appendix Tables.

A. Introduction

This document focuses on exposures to cannabis (marijuana) smoke and to delta-9-tetrahydrocannabinol (Δ^9-THC; (-)-(6aR,10aR)-6,6,9-trimethyl-3-pentyl- 6a,7,8,10a-tetrahydro-6H-benzo[c]chromen-1-ol), and reviews the scientific evidence on the developmental toxicity of cannabis smoke and Δ^9-THC. Δ^9-THC is a constituent of cannabis smoke, as well as a constituent of plants in the genus *Cannabis*. In this document, the terms 'cannabis' and 'marijuana' are used interchangeably.

In California, use of cannabis for physician-recommended purposes has been legal since 1996. As of January 1, 2018, the Medicinal and Adult-Use Cannabis Regulation and Safety Act legalized adult use of cannabis in California. The law requires the following statement be given on pre-roll cannabis cigarettes and packaged flower products:

> "Cannabis use while pregnant or breastfeeding may be harmful".[5,6]

Identity of Cannabis Smoke and Δ^9-THC

Cannabis has been used for medicinal and psychotropic purposes in some parts of the world for thousands of years. Inhaling cannabis smoke for its psychotropic properties became popular in western cultures in the twentieth century. Cannabis smoke is formed when the dried flowers, leaves, stems, seeds and resins of plants in the genus *Cannabis* are burned. Cannabis smoke aerosol contains thousands of organic and inorganic chemicals, including psychoactive cannabinoids, which are unique to *Cannabis* plants.

The following is a list of common cannabis plant products that are smoked:

- Bud. The flower tops of unpollinated female cannabis plants. Buds have the highest Δ^9-THC content of all parts of the plant. The bud is probably the most common form of cannabis smoked currently in the US.
- Ganja. A mixture of flowering tops and leaves from female cannabis plants, dried and diced, or powdered. Other terms used for ganja include: kif, kief, kef, keef (Morocco and Algeria); tekrouri, takrouri (Tunisia); and dagga (southern Africa).
- Hash. Crude resin from flowering tops of unfertilized female cannabis plants. Processed differently in different parts of the world. Often collected by rubbing

[5] Business and Professions Code section 26120(c)(1).

[6] California Code of Regs., Title 17, section 40404.

onto hands, cloth, or leather jackets, or by sifting. Compressed into blocks. Other terms used for hash include: Hashish (Middle East) and charas (Far East).

- Leaf. Leaves of the marijuana plant. Less potent than buds or flower tops with regard to Δ^9-THC content, leaves were commonly smoked in the US in the 1960s and 1970s.
- Bhang. Generally prepared from the buds and leaves of male plants. Most often used for making beverages but sometimes smoked. The term bhang originates from India and Bangladesh.
- Sinsemilla. Form of cannabis produced by cloning the female plant that develops flowers with few or no seeds. The result is a more potent cannabis.

Cannabis smoke contains several thousand different compounds (Sparacino et al. 1990). Some of these compounds are released unchanged from the plant material as it burns, and others are products of either pyrolysis or incomplete combustion. Cannabis smoke consists of some chemicals present in the gas phase, some present in particulate matter, and some semi-volatile compounds that transition between the gas and particulate phases. Cannabis smoke includes a large variety of organic and inorganic chemicals, including amines, aromatic amines, aza-arenes, polycyclic aromatic hydrocarbons (PAHs), carbonyls, phenolics, pyrazines, pyrimidines, pyrroles, pyridines, isoxazoles, metals (arsenic, cadmium, chromium, lead, nickel, and selenium), hydrogen cyanide, carbon monoxide (CO), nitric oxide (NO), other nitrogen oxides (NO_x), ammonia, and over 60 cannabinoid compounds (Hoffmann et al. 1975; Lee et al. 1976; Moir et al. 2008; Sparacino et al. 1990).

Cannabis smoke and tobacco smoke share many characteristics with regard to chemical composition and toxicological properties. Tobacco smoke has been listed under Proposition 65 as causing reproductive toxicity (developmental, male, female endpoints) since 1988. Individual constituents present in both cannabis smoke and tobacco smoke are also listed under Proposition 65 list as causing reproductive toxicity, including: 1,3-butadiene (developmental, female, male), cadmium (developmental, male), carbon monoxide (developmental), hydrogen cyanide (male), lead (developmental, female, male), mercury (developmental, female, male), methanol (developmental), and toluene (developmental).

The cannabinoid compounds present in plants in the genus *Cannabis* are terpenophenolic compounds, commonly containing 21 carbons. The major cannabinoids present in cannabis smoke are:

- Δ^9-THC, the most potent psychoactive compound present in cannabis (ElSohly 2002)
- Δ^8 - THC

- cannabinol (CBN)
- cannabidiol (CBD)
- cannabichromine, and
- 11-OH-Δ^9-THC.

In the past, the levels of Δ^9-THC in cannabis smoked in the US typically ranged from 1 to 3%. However, over the last 30 years, levels of Δ^9-THC have been increasing as a result of the selective cultivation of plants. Typical levels of Δ^9-THC in cannabis are now greater than 6%. The addition of hashish oil (a cannabinoid-rich extract from *Cannabis* plant material) to the dried material can boost Δ^9-THC levels even higher (e.g., 20%).

Five major studies (Gieringer et al. 2004; Hoffmann et al. 1975; Lee et al. 1976; Moir et al. 2008; Sparacino et al. 1990) have been conducted to identify the major constituents of the thousands of chemicals present in cannabis smoke, as follows:

- Moir *et al*. (2008) used standardized cannabis, which was harvested in May 2004 and produced by Prairie Plant Systems Inc., of Saskatoon, Canada, for Health Canada. The material tested consisted of flowering heads only (reference: H55-MS17/338-FH). Smoke was generated using a smoking machine, operating under two different smoking conditions. The first smoking condition involved a puff volume of 35 milliliters (ml), a puff duration of two seconds, and a puff interval of sixty seconds, while the second smoking condition, referred to as 'extreme,' involved a puff volume of 70 ml, a puff duration of two seconds, and a puff interval of 30 seconds.
- Gieringer *et al*. (2004) used standard National Institute on Drug Abuse (NIDA) cannabis obtained from an independent laboratory. The mean Δ^9-THC content was 4.15%. Smoke was generated by combusting the cannabis in a glass pipe bowl, and collected in a volatile gas trap.
- Sparacino *et al.* (1990) generated cannabis smoke from two samples of Mexican cannabis, one with a "low" Δ^9-THC content (1.3%) and another with a "high" Δ^9-THC content (4.4%). Smoking machines employed either a constant draft apparatus, or an intermittent puff smoking system.
- Hoffmann *et al*. (1975) analyzed cannabis leaves obtained from the Division of Cancer Cause and Prevention of the National Cancer Institute (NCI). The NCI material was prepared from confiscated cannabis, grown in Mexico. Smoke was generated using a smoking machine.
- Lee *et al*. (1976) obtained Mexican cannabis containing 2.8% Δ^9-THC from the National Institute of Mental Health, in Rockville, Maryland, and generated smoke using a smoking machine under conditions simulating that of an average tobacco cigarette smoker.

Approximately 350 different chemicals have been identified by these investigators and they are listed below.

acenaphthene
acenaphthylene
acetaldehyde
acetamide
acetone
8-acetoxy-pyrazolobenzo-as-triazine
3-acetylpyridine
acrolein
acrylonitrile
alkyl nitrile
aminobenzamide
3-aminobiphenyl
4-aminobiphenyl
aminodimethylpyrimidine
aminodiphenylene oxide
aminomethylquinoline
1-aminonaphthalene
2-aminonaphthalene
m-aminophenol
aminoquinoline
β-amiryn
ammonia
anthanthrene
anthracene
arsenic
1-azidonaphthalene
1,2,3,3a,4,5,6,7, 5-azulenemethanol
benz[a]anthracene
benzacenaphthylene
benzene
benzeneacetonitrile
1,2-benzenedicarboxylic acid, bis (2)1,2-benzenediol
1,3-benzenediol, 2-(3,7-dimethyl-2
benzimidazole
benzo[a]fluorene
benzo[a]pyrene
benzo[b]fluoranthene
benzo[b]fluorene
benzo[c]fluorene
benzo[e]pyrene
benzo[g,h,i]perylene
benzo[j]fluoranthene
benzo[k]fluoranthene
benzofluoranthene
benzofuran
2H-1-benzopyran-5-ol, 2-methyl-2-(41,4-benzoquinone
benzyl acetate
benzyl acetophenone
N-benzyl-4-aminobutyronitrile
binaphthyl
α-bisabolol
1,3-butadiene
1-butoxy-2-propanol
tert-butyl-parahydroxybenzoate
butyraldehyde
butyroamide
cadmium
caffeine
DL-cannabichromene
cannabinol (CBN)
carbazole
β-carboline
carbon monoxide (CO)
caryophyllene
caryophyllene oxide
catechol
1-chloro-octadecane
cholesta-3,5-dien-7-one
cholesterol
cholesteryl acetate
chromium
chrysene
m,o,p-cresol
crotonaldehyde
p-cumyl phenol
cyclododecane
cyclohexadecane
4H-cyclopenta[d,e,f] phenanthrene
cyclopentadiene
1a,2,3,1H-cyclopropa [a]naphthalene
cyclopropanenanoic acid, 2-[(2-bu
4,7,10-cycloundecatriene
decahydro-4a-methyl-1-naphthalene
1-decanol
1-decene
dibenz[a,h]anthracene
dibenz[a,i]anthracene
dibenz[a,i]pyrene
dibenzo[a,e]pyrene
dibenzofuran
d-dibenzopyrene
dibutyl phthalate
diethyl biphenyl
2,2'-diethyl-1,1'-biphenyl
diethylnitrosamine
diethylphenylene diamine
1,2-dihydro-3-isobutyl-1-methylpyrazine-2-one
2,3-dihydrobenzofuran
dihydroxymethyl phenyl quinazoline
2,3-dihyroxyprohexadeacanoic acid
dimethoxybenzene isomer
dimethyl naphthyridine
dimethyl tetrazine
7,11-dimethyl-1,6,10-dodetatriene
dimethylbenzimidazole
3,4-dimethylbenzoic acid
3,3-dimethylcyclobutane-carbonitrile
10,10-dimethylenebicyc
dimethylethanamide imidazole
dimethylethylpyrrole
1-(1,5-dimethylhexyl) cyclohexane
1,2-dimethylimidazole
N,N-dimethyl-N-(p-methoxyphenyl) formamide
N,N'-dimethyl-N,N'-diethyl-p-phenylene diamine
dimethylnaphtho(2,3,6) thiophene
dimethylnaphthyridine
dimethylnitrosamine
3,3-dimethyloxetase
2,4-dimethylphenol
2,5-dimethylphenol
dimethylpiperazine
dimethylpyrimidone
2,4-dimethylquinazoline
dimethyltrisulfide
dimethyl-β-carboline isomer
dioctyl phthalate
diphenylamine
diphenylethyne
diphenylpyridine isomer
2,6-diterbutylnaphthalene
ditolyl ethane
docosane
2-dodecen-1-yl (-)succinic anhydride
5-dodecyldihydro-2 (3H)-furanone
dronabinol (THC)
eicosane
(E)-3-eicosene
3-eicosene
ethoxy benzaldehyde
ethoxyquinazoline
ethyl hydroxyl acetophenone
ethyl-4H-cyclopenta [d,e,f]phenanthrene
ethylbinaphthyl
ethylindole
ethylmethylbiphenyl
ethylphenol, 4-
fluoranthene
fluorine
formaldehyde
glaucyl alcohol
heneicosane
henricosyl formate, 1-
heptacosane
heptadecane
2-heptadecanol
2-heptadecanone
hexacosane
hexadecanal
hexadecanamide
hexadecane
(Z)-3-hexadecane
hexadecanoic acid
hexadecanoic acid, hexadecyl ester
1-hexadecanol
2-hexadecanol
n-hexadecanol
cis-11-hexadecen-1-yl acetate

9-hexadecenoic acid eicosyl
9-hexadecenoic acid eicosyl ester
hexanedioic acid dioctyl ester
hexanenitrile 3(pyrrolidnyl-methylene)
2-hexyl-1-decanol
hydrogen cyanide
hydroquinone
5-hydroxyindole
hydroxymethylquinoline
4,5,6,7-1H-indazole
indeno[1,2,3,-c,d]pyrene
indole
isoprene
lead
2-p-mentha-1,8-dien-3-y resocinol
mercury
1H-3a,7-methanoazulene, octahydro-1
methanol
methoxy propyl pyrazine
2-methoxy-3-methylpyrazine
methoxybenzaldehyde
methyl acetyl pyrrole
methyl benzimidazole
3-methyl benzoic acid
4-methyl carbostyril
methyl ethyl ketone
methyl ethyl pyrazine
methyl ethyl pyrrole
1-methyl imidazole
methyl palmitate
methyl phenyl cinnoline
methyl pyridine carboxylic acid
methyl pyrimidine
methyl stearate
16-methyl-, met heptadecanoic acid
2-methyl-1,4-benzenedoil
3-methyl-1,8-naphthyridine
2-methyl-1-hexadecanol
1-methyl-1H-indene
3-methyl-1H-indole
4-methyl-1H-indole
N-methyl-2-pyridinamine
1-methyl-4-(5-methyl-1-cylohexene
3-methyl-4-ethylpyrrole
3-methyl-5-triazolo(4,3-a)pyrazine
methylacenaphthylene
methylaminonaphthyridine
1-methylanthracene
2-methylanthracene
10-methylbenz[a]anthracene
2-methylbenz[a]anthracene
3-methylbenz[a]anthracene
4-methylbenz[a]anthracene
5-methylbenz[a]anthracene
6-methylbenz[a]anthracene
8-methylbenz[a]anthracene
9-methylbenz[a]anthracene
methylbenzoxazole
methylbinaphthyl
methylcarbazole
1-methylchrysene
2-methylchrysene
3-methylchrysene
5-methylchrysene
6-methylchrysene
N-methyldiphenylamine
methylethylnitrosamine
1-methylfluoranthene
2-methylfluoranthene
3-methylfluoranthene
7-methylfluoranthene
8-methylfluoranthene
1-methylfluorene
2-methylfluorene
2-methylfuran
3-methylheneicosane
methylindole
methyl-n-(pyrid-2-yl) dihydropyrrole
N-methyl-N-[4-[4-4-methoxy acetamide
N-methyl-N-[4[4-methoxy-acetamide
1-methylnaphthalene
2-methylnaphthalene
1-methylphenanthrene
2-methylphenanthrene
3-methylphenanthrene
9-methylphenanthrene
1-methylphenazine
methylphenyl quinoxaline
methylpropionyl furan
methyl-pteridinone isomer
methylpyrazine
1-methylpyrene
2-methylpyrene
4-methylpyrene
methylpyriloindole
methylquinoline
methylthiazolopyrimidine
methylthiopyridine
1-methyl-β-carboline
naphthalene
naptho-sydinone
nickel
nitric oxide (NO)
nitroacetanilide
nitrogen oxides (NOx)
nitropicoline
nonacosane
nonadecane
nonadecene
1-nonadecene
octacosane
octadecane
1-octadecanethiol
2,3-octadecanoic acid, dihydroxypro
1-octadecene
5-octadecene
1,2,3,5,6,7,8,8a-octanaphthalene
1-octdecanethiol
6-octen-1-ol, 3,7-dimethyl acetate
1,1'-oxybis-octane
pentacosane
pentadecane
pentadecanoic acid
1-pentadecene
pentyl cannabinol, 3-n-
3-n-pentyl-delta-9-tetrahydrocannabinol
perylene
phenanthrene
1,2,1-phenanthrenecarboxylic acid
1-phenantthrenecarboxylic acid, 7-et
phenoxy ethanol
N-phenyl acrylamide
phenyl alcohol
phenyl benzothiazole
1-phenyl decane
phenyl methyl quanidine
phenyl methyl urea
phenyl pyrazoline
phenyl pyridine
phenyl urea
phenylbenzimidazole
(α-picolidene)-n-propylamine, N-
α-picoline
2-pmemtha-1,8-dien-3-y-resorcinol
propionaldehyde
propionamide
2-(propylamino)benzothiazole
propylbenzimidazole
pyrene
pyridine
quaterphenyl
quaterphenyl diphenyl-acenaphtylene
quinoline
resorcinol
selenium
squalene
styrene
tetracosane
tetradecanoic acid
2- (tetradecyloxy)-ethanol
Δ,8-tetrahydrocannabinol
Δ,9-tetrahydrocannabinol
tetramethylcyclopentanedione
2,6,10,14-tetramethyl-hexadecane
3,5,6,7-tetra-s-indacen-1(2H)-one
2,3,5,6-tetra-s-indacene-1,7-dione
2-thiocyanatodiphenylamine
toluene
tolyl azide
tricosane
(Z)-9-tricosene
1,7,11-trimethyl cyclo-tetradecane
trimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine
trimethylnaphthyridine
2,2,4-trimethylpenta-1,3-diol-di-isobutyrate
1,3,5-trimethylpyrazole
2,6,10-trimethyl-tetradecane
tropolone
1-undecanol
valeramide
2-vinyl pyridine
vitamin E

Exposure and Use Information

Exposure to cannabis and/or the active chemical compounds in cannabis can occur through:

- smoking – combusting the cannabis or cannabis mixture and inhaling the smoke.
- vaping and other vaporization methods – heating cannabis or cannabis extracts to temperatures below the combustion point of approximately 230°C, that result in formation of a vapor, and inhaling the vapor.
- dabbing - heating highly concentrated cannabis or hashish to form a vapor), and
- ingesting cannabis or cannabis extracts (Hasin 2018).

In the US, the popularity of cannabis, as measured by first-time use rates, increased greatly in the late 1960s, reached a plateau in the 1970s, dropped to a mid-level in the 1980s, and increased again through the 1990s.

Data from the US National Survey on Drug Use and Health (NSDUH) obtained from 2002 – 2014 were included in several studies that reported increasing trends in cannabis use in the US population during this period (Brown et al. 2017; Carliner et al. 2017; Coleman-Cowger et al. 2017; Ko et al. 2015; Mauro et al. 2018).

The prevalence of past-month cannabis use was highest among women age 18–25 years, reaching 7.47% (95% CI, 4.67–11.93) in 2014.

- In non-pregnant women age 18–25 years, past-month use in 2014 was 9.27% (95% confidence Interval (CI), 8.90–9.65) and past-year use in 2014 was 15.93% (95% CI, 15.48–16.40), with similar trends over time. (Brown et al. 2017; Ko et al. 2015).

- Among pregnant women, past-month cannabis use increased from 2.37% (95% CI, 1.85–3.04) in 2002 to 3.85% (95% CI, 2.87–5.18) in 2014 (Brown et al. 2017). The same study reported that past-year cannabis use among pregnant women increased from 8.64% (95% CI 7.32, 10.19) in 2002 to 11.63% (95% CI 9.78, 13.82) in 2014.

In Northern California, a study of pregnant females from a large integrated health care system (approximately 4 million patients) reported cannabis use from self-report and urine testing (Young-Wolff et al. 2017). In this population, cannabis use was found to increase from 4% to 7% from 2009 to 2016. A recent update with the self-report data for 2017 from this population suggests that cannabis use among pregnant women continues to increase, approximately 6.5% per year (i.e., annual relative rate of change of 1.065). In 2016, the percentage of pregnant females using cannabis was highest among those younger than 18 years, at 22%, followed by those 18-24 years of age, at 19%. Results from the update also show that among women using cannabis in the year

before or during pregnancy, the percentage of women who reported daily cannabis use increased the most, 11% per year (annual relative rate of change approximately 1.11). (Young-Wolff et al. 2019b).

OEHHA does not have information regarding whether recreational use of cannabis has increased since the January 1, 2018 legalization of adult use in California.

Literature Search and Screening Methods

A search of the literature on the developmental toxicity of cannabis smoke and Δ^9-THC was conducted by OEHHA librarian Nancy Firchow, MLS. The goal was to identify peer-reviewed open source and proprietary journal articles, print and digital books, reports and gray literature that potentially reported relevant toxicological and epidemiological information on the developmental toxicity of cannabis smoke and Δ^9-THC. The search sought to identify all literature relevant to the assessment of evidence on developmental toxicity.

Search Process

PubMed MeSH browser was used to identify subject headings, other index terms and synonyms for cannabis and for concepts related to reproduction and development. Preliminary searches were run and results evaluated to identify additional relevant search terms. The resulting search strategy was executed in PubMed. (See Appendix 3.1 for the detailed PubMed search strategy.)

The PubMed strategy was then tailored for use in additional databases and data sources, listed below, according to the search interface and features unique to each resource. For instance, MeSH terms were replaced with Emtree terms for the Embase search strategy.

Additional targeted searches were performed in PubMed and other resources as needed to expand retrieval on specific aspects of the subject. Relevant literature was also identified from citations in individual articles, and through alert services (e.g. ScienceDirect, Google Scholar etc.).

Data Sources:

The following is a list of the major data sources searched to find information on cannabis.

Biomedical literature databases

- PubMed (National Library of Medicine) (https://www.ncbi.nlm.nih.gov/pubmed)
- Embase (https://www.embase.com)
- Scopus (https://www.scopus.com)
- TOXLINE (National Library of Medicine): Toxicology Literature Online (https://toxnet.nlm.nih.gov/newtoxnet/toxline.htm)
- Toxnet DART (Developmental and Reproductive Toxicology Database) (https://toxnet.nlm.nih.gov/newtoxnet/dart.htm)

Other Databases and Web Resources

- National Toxicology Program (https://ntp.niehs.nih.gov/)
- US EPA IRIS (https://www.epa.gov/iris)
- Agency for Toxic Substances and Disease Registry (ATSDR) (https://www.atsdr.cdc.gov/)
- INCHEM (http://www.inchem.org/)
- National Academies of Sciences, Engineering and Medicine (https://www.nap.edu/)
- World Health Organization IRIS (http://apps.who.int/iris/)
- Google Scholar (https://scholar.google.com/schhp?hl=en-US).
- OEHHA (https://oehha.ca.gov/).

Table A.1. Number of studies identified in the process by the OEHHA librarian*.

	PubMed	Embase	Scopus	Toxnet	Toxnet DART	Gray literature	Total
Primary Search – Human & Animal (11.8.2018)	2907	1349	---	---	459	4	4719
Human only – 1.28.19	2241	931	---	---	---	---	3172
Neurodevelopmental toxicity reviews – 2.21.2019	137	---	---	2	---	---	139
Epigenetics – 2.26.2019	200	385	187	---	---	---	772
Mechanisms of Action reviews 4.2.2019	35	---	---	---	---	---	35
Mechanisms preimplantation/implantation 4.25.2019	74	---	---	---	---	---	74
PubMed – 5.16.2019	245	117	---	---	---	---	362
Total:							9,273

*Number of references are index by time of search and database source

Screening Process

Citations retrieved from literature searches were uploaded to EndNote libraries and duplicates were removed. The EndNote libraries were then uploaded to SWIFT Active Screener Review (https://www.sciome.com/swift-review/) for multi-level screening using specific inclusion and exclusion criteria.

In Level 1 screening, citations were reviewed independently by two OEHHA staff, based solely on study titles and abstracts, to eliminate studies or articles that do not contain information on the developmental toxicity of cannabis or Δ^9-THC and any of the key related topics, such as epidemiology, metabolism, mechanism of action, etc. The initial screen was intended to retrieve all studies deemed to have a reasonable possibility of containing information that could be useful for the review process. A positive response by only one of the reviewers was not sufficient to pass a publication on to the next review level. In that case, the controversy was resolved as soon as possible so the screening software can continue with the algorithm by selecting the appropriate citations.

In Level 2 screening, the full text was obtained for all references that passed the Level 1 review. These full papers were screened independently by one OEHHA staff, using similar inclusion/exclusion criteria as was used in the Level 1 screening. However, Level 2 reviewers could make more accurate judgements about the relevance of the articles because they were reviewing the full text in addition to the title and abstract.

Level 1 and 2 screenings were repeated and SWIFT search results were updated with additional relevant studies identified from the bibliographies of the original set of references ("secondary citations").

Table 1. summarizes the results of OEHHA's systematic review of the literature on the developmental toxicity of cannabis. A total of 9273 unique references were identified through the OEHHA's librarian search process. Studies were reviewed for whether the information was relevant to this evaluation. In addition to the studies provided by the OEHHA librarian, other undetermined number of studies were obtained as a result of current reading of primary studies available, process known as "snow ball" search.

PubMed Search Strategy

The search was executed in PubMed (https://www.ncbi.nlm.nih.gov/pubmed) on November 8, 2018. In order to focus the search, Cannabis MeSH terms were restricted to [major] and Cannabis text terms were restricted to [title]

- Development and reproductive toxicity (DART) terms were adapted from the search strategy used to create the PubMed Developmental and Reproductive Toxicology search filter (https://www.nlm.nih.gov/bsd/pubmed_subsets/dart_strategy.html)
- No date or language limits were applied

The search strategy is outlined below in Table A.2.

Table A.2 PubMed Search Strategy

Set	Search Terms	Notes
1	(cannabis[Majr] OR cannabis[ti] OR marijuana[ti] OR marihuana[ti] OR medical marijuana[Majr] OR Marijuana Smoking[Majr] OR marijuana abuse[Majr] OR hashish[ti] OR hash-oil[ti] OR cannabinoids[Majr] OR Cannabinoid*[ti] OR Delta-9-tetrahydrocannabinol[ti] OR Δ9-tetrahydrocannabinol[ti] OR THC[ti] OR 8- tetrahydrocannabinol[ti] OR Cannabichromene[ti] OR Cannabicyclol[ti] OR Cannabielsoin[ti] OR Cannabigerol[ti] OR Cannabidiol[majr] OR Cannabidiol[ti] OR Cannabinol[majr] OR Cannabinol[ti] OR Cannabitriol[ti] OR Dronabinol[ti])	Cannabis terms
2	(17-alpha-hydroxypregnenolone[tiab] OR 17-alpha-hydroxyprogesterone [tiab] OR 17-beta-hydroxysteroid dehydrogenase[tiab] OR 17-beta-estradiol [tiab] OR Abortion*[tiab] OR Abortion, spontaneous[majr] OR Adrenarche[tiab] OR Androgen antagonists[majr] OR Androgens[majr] OR Androgen*[tiab] OR Androstenedione[tiab] OR Anogenital distance[tiab] OR Ano-genital distance[tiab] OR Anovulat*[tiab] OR Aspermia[tiab] OR Azoospermia[tiab] OR Behavior, animal[majr] OR Birth defect*[tiab] OR Birth weight[majr] OR Birth weight[tiab] OR Breast feeding[majr] OR Breast feed*[tiab] OR breastfeed*[tiab] OR Breast[majr] OR Conception*[tiab] OR Congenital abnormalities[majr] OR Congenital[tiab] OR Copulat*[tiab] OR Defect[tiab] OR defects[tiab] OR defective[tiab] OR Developmental[tiab] OR DNA Damage[majr] OR Ejaculat*[tiab] OR Embryo*[tiab] OR Embryoes[tiab] OR Embryonic[tiab] OR Embryotoxic*[tiab] OR “Embryonic and fetal development”[majr] OR Embryonic Structures [majr] OR Endometri*[tiab] OR Epididym*[tiab] OR Erecti*[tiab] OR Estradiol [tiab] OR Estrogen antagonists[majr] OR Estrogen receptor modulators[majr] OR Estrogens[majr] OR Estrogen*[tiab] OR Estrus[tiab] OR Fecund*[tiab] OR Fertilization[tiab] OR Fertility[tiab] OR Fetal[tiab] OR Fetus[majr] OR Fetus[tiab] OR foetal[tiab] OR foetus[tiab] OR Follicle stimulating hormone[tiab] OR FSH[tiab] OR Genetic diseases, inborn[majr] OR Genital diseases, female[majr] OR Genital diseases, male[majr] OR Genitalia [majr] OR Genital*[tiab] OR Germ cells[majr] OR Germ cell*[tiab] OR Gestat*[tiab] OR Gonad*[tiab] OR Gonadal disorders[majr] OR Gonadal hormones[majr] OR Gonadotropins[majr] OR Gonads[majr] OR Human development[majr] OR Implantation[tiab] OR Intrauterine[tiab] OR In utero[tiab] OR Infant*[tiab] OR Infant, newborn[majr] OR Infertile*[tiab] OR Inhibin[tiab] OR Lactation disorders[majr] OR Lactat*[tiab] OR Luteinizing hormone[tiab] OR LH[tiab] OR Mammary[tiab] OR Mammary glands, animal[majr] OR Maternal exposure[majr] OR Maternal[tiab] OR Mating[tiab] OR Menses[tiab] OR Menstrua*[tiab] OR Miscarriage*[tiab] OR neurobehav*[tiab] OR neurodevel*[tiab] OR Oligospermia[tiab] OR Oocyte*[tiab] OR Ovari*[tiab] OR Ovary*[tiab] OR Ovulat*[tiab] OR Ovum[tiab] OR Paternal exposure[majr] OR Paternal[tiab] OR Peripubert*[tiab] OR Pituitary hormones[majr] OR Placenta*[tiab] OR Placental hormones[majr] OR Preconception*[tiab] OR Pre-conception*[tiab] OR pregnancy[mh] OR Pregnan*[tiab] OR Pregnancy complications[majr] OR Premature*[tiab] OR Prenatal exposure delayed effects[majr] OR Prenatal[tiab] OR Pre-natal[tiab] OR Preterm[tiab] OR Pre-term[tiab] OR Progestins[majr] OR Progestin*[tiab] OR Progesterone[tiab] OR Prostat*[tiab] OR Reproduct*[tiab] OR Reproductive physiological phenomena[majr] OR Semen*[tiab] OR Semen[majr] OR Seminiferous[tiab] OR Seminal[tiab] OR Sexual development[majr] OR Sperm[tiab] OR spermato*[tiab] OR Sterile[tiab] OR Sterility[tiab] OR Stillbirth*[tiab] OR Stillborn[tiab] OR Teratogen*[tiab] OR Teratogens[majr] OR Testic*[tiab] OR Testis[tiab] OR Testosterone[tiab] OR Thyroid[tiab] OR Thyroid hormones[majr] OR Urogenital abnormalities[majr] OR Urogenital*[tiab] OR Uterine[tiab] OR Uterus[tiab] OR Vagina*[tiab] OR Zygote[tiab])	DART terms
3	#1 AND #2	Combine Cannabis & DART
4	((neuropsych*[tiab] OR stemi[tiab] OR pain[tiab] OR sleep apnea*[tiab] OR lennox-gastaut[tiab] OR epilep*[tiab] OR hiv[tiab] OR human immunodeficiency virus[tiab] OR seizure*[tiab]) NOT (prenatal[tiab] OR perinatal[tiab] OR maternal[tiab] OR paternal[tiab] OR in utero[tiab] OR pregnan*[tiab] OR pregnancy[mh] OR fetus[tiab] OR fetus[mh] OR foetus[tiab] OR fetal[tiab] OR foetal[tiab] OR embryo*[tiab] OR reproducti*[tiab] OR breastfeed*[tiab] OR breast-feed*[tiab] OR developmental[tiab]))	Terms not of interest unless they appear with specific DART terms

Set	Search Terms	Notes
5	#3 NOT #4	Remove terms not of interest -- Final results

B. Pharmacokinetics

Cannabis smoke is a complex aerosol mixture of thousands of chemicals present in the gas and particulate phases (Huestis 2005; Sparacino et al. 1990). The available information on the pharmacokinetics and metabolism of this complex mixture is limited. Here we summarize the available information on cannabis smoke pharmacokinetics. We also summarize information on the pharmacokinetics and metabolism of Δ^9-THC from exposures to a variety of cannabis-derived sources (e.g., cannabis, cannabis smoke, cannabis extracts, decoctions, vaporization of cannabis or cannabis extracts, Δ^9-THC) by various exposure routes (inhalation, ingestion, intravenous injection).

Absorption

Smoke

In the process of cannabis smoke inhalation, the aerodigestive tract - lips and mouth tissues, tongue, nose, throat, vocal cords, and portions of the esophagus and trachea - and the lungs are directly exposed to cannabis smoke components. Absorption of the gaseous constituents may occur at multiple sites within the aerodigestive tract and the lungs, and is dependent upon solubility and vapor pressure. The particle size determines where deposition will occur in the aerodigestive tract and lungs.

Wu *et al*. (1988) determined that 80.7 to 86.7% of the inhaled resinous total particulate matter (i.e., tar) in cannabis smoke would be deposited in the lung. Some particles deposited within the aerodigestive tract will enter the gastrointestinal tract, as will some particles deposited in the lungs, which will then be cleared by mucociliary transport. Thus, while the principal sites of absorption are the lungs and the aerodigestive tract, some absorption of cannabis smoke components is expected to occur via the gastrointestinal tract (Wu et al. 1988).

By way of leaching and dissolution, some of the adsorbed chemicals on smoke particles will traverse the plasma membrane and gain entry into epithelial cells and other cells of the lungs. Many chemicals in cannabis smoke are readily absorbed, including the cannabinoids.

Pharmacokinetic studies of cannabis smoke in humans have shown that cannabinoids are quickly absorbed from the lungs, reaching peak plasma levels within seven to eight minutes. Δ^9-THC is detectable in blood seconds after the first puff of a cannabis cigarette (Milman et al. 2012; Musshoff and Madea 2006). In a study by Huestis *et al*. (1992a), Δ^9-THC plasma concentrations were measured in six individuals following exposure to a single cannabis cigarette, containing either 1.75 or 3.55% Δ^9-THC. Measured plasma Δ^9-THC concentrations after a single inhalation (puff) were 7.0±8.1 ng/ml and 18.1±12.0 ng/ml, respectively. Plasma Δ^9-THC concentrations measured

after smoking the entire cigarette peaked at 84.3 ng/ml for the lower Δ^9-THC content cigarette, and 162.2 ng/ml for the high Δ^9-THC content cigarette (Huestis et al. 1992a).

Studies have shown that the bioavailability within the lungs of Δ^9-THC and other cannabinoids present in cannabis smoke can vary considerably, depending upon the burning characteristics of the cannabis cigarette, the depth of inhalation, the inhalation volume, and the holding time (Chiang and Rapaka 1987; Huestis 2007). The bioavailability of other constituents of cannabis smoke would also be expected to vary depending upon these same factors.

Δ^9-THC present in cannabis smoke is readily absorbed from the oral cavity (Huestis and Cone 2004).

Second-hand smoke

Second-hand or passive exposure to cannabis smoke was investigated in a study in the Netherlands with ten volunteers that were non-cannabis smokers. After 3 h of exposure to second-hand cannabis smoke in a local café, Δ^9-THC was detectable (> 2 ng/mL) in the oral fluid of seven of the 10 participants. Similarly, in rats exposed for 50 min to second-hand smoke, Δ^9-THC in plasma was detected immediately after cessation of exposure (Ravula et al. 2018).

Vapor

Absorption of Δ^9-THC from vaporized cannabis, as indicated by detection in blood and plasma, has been demonstrated following inhalation of vapors produced by heating cannabis to temperatures of approximately 200°C (Abrams et al. 2007; Hartman et al. 2015a; Newmeyer et al. 2017a; Spindle et al. 2019). This temperature, which is below the burning point of cannabis (230°C); produces vapors which can then be inhaled.

Cigarettes from the National Institute on Drug Abuse (NIDA) were vaporized by heating the cannabis material to about 200°C. This technique of administration results in the same area under the curve (AUC) for Δ^9-THC as smoking cannabis does (Hartman et al. 2015a). However, compared to smoking, inhaling the vapor resulted in higher plasma concentrations of Δ^9-THC for the first hour after exposure. Plasma concentrations of Δ^9-THC were similar between the two exposure methods for the next five hours (Abrams et al. 2007). These observations are in agreement with a more recent study (Spindle et al. 2019). In this study, Δ^9-THC and three of its metabolites (11-OH-THC, THC-COOH, and THC-COOH glucuronide) were all detected in blood and oral fluid in the first 10 min of exposure, regardless of exposure to cannabis smoke or vapor. The Δ^9-THC and metabolite distribution appears similar between the two methods of exposure; however, vaporization resulted in higher initial concentrations of Δ^9-THC and its metabolites (Spindle et al. 2019).

Oral

There are a number of studies that have examined the oral absorption of cannabis following ingestion. These studies most often heated ground cannabis plant material to 121°C for 30 min to release a carboxylic group from monocarboxylic acids of Δ^9-THC that are present in the plant, after which the heated product was used in cooking. In male volunteers that consumed brownies containing cannabis, Δ^9-THC and THC-COOH were detected in urine samples for up to 5 – 6 days after exposure (Cone et al. 1988), or in blood for up to 48 hours, with the time of elimination from the blood dependent on the individual's past history of cannabis consumption (Newmeyer et al. 2017b). Volunteers exposed to a decoction of cannabis plant material containing Δ^9-THC in water or milk had different blood levels of Δ^9-THC or metabolites, depending on the liquid used for decoction. After decoction in water only trace amounts of Δ^9-THC and 11-OH-THC were detected in blood samples. After milk decoction, Δ^9-THC and 11-OH-THC were detected in blood for up to 10 h, at levels approximately 10-fold higher than was seen with the water decoction. Following consumption of the milk decoction, THC-COOH was also detected in blood, with the maximum concentration occurring between 1 and 3 h; it remained detectable in whole blood for more than 50 h (Giroud et al. 2000).

Distribution

The distribution of Δ^9-THC present in cannabis smoke has been investigated in animal studies (Hunault et al. 2010; Poklis et al. 2010). Δ^9-THC is lipophilic and the majority absorbed is distributed to highly vascularized tissues such as the brain. The concentration of Δ^9-THC in the brain is similar to that in plasma, suggesting that transport of Δ^9-THC into the brain is not hindered by the blood-brain barrier. Δ^9-THC is sequestered in body fat, with only a small fraction present in the blood. In blood, Δ^9-THC is extensively bound to plasma protein (97% to 99%) (NTP 1996).

Studies in non-human primates, dogs and sheep have shown that Δ^9-THC crosses the placenta and reaches the fetus (Abrams et al. 1985; Bailey et al. 1987; Martin et al. 1977). The Δ^9-THC levels present in fetal tissues (brain and liver) and plasma were lower than those in maternal tissues (Bailey et al. 1987; Lindgren 1983). In humans, it was shown that Δ^9-THC crosses the placenta and is also present in breast milk (Grotenhermen 2003). The two main metabolites of Δ^9-THC, 11-OH-THC and THC-COOH, have been detected in umbilical cord tissue (Chittamma et al. 2013; Kim et al. 2018; Toennes et al. 2018).

A pharmacokinetic model describing the disposition of Δ^9-THC in occasional cannabis smokers predicts that there will be a detectable plasma Δ^9-THC concentration (1.0 ng/ml) for up to 8h (Marsot et al. 2017).

Δ^9-THC has been detected in oral fluid up to 8 days after smoking (Andas et al. 2014) and levels were found to be correlated with an individual's past history of cannabis

consumption (e.g., extent and frequency of use) (Swortwood et al. 2017). After exposure to cannabis by smoking, vaporization or ingestion, Δ^9-THC, 11-OH-THC, THC-COOH, and other cannabis metabolites were detected in the oral fluid of healthy volunteers that were frequent cannabis smokers, regardless of the method of exposure. In healthy volunteers that were occasional cannabis smokers, Δ^9-THC and THC-COOH, but not 11-OH-THC, were detected after each of the three methods of exposure.

Metabolism

The chemical constituents of cannabis smoke can be metabolized by tissues of the aerodigestive tract and the lungs, as well as the blood and other organs subsequent to systemic distribution. A variety of Phase I and Phase II enzymes are expected to be involved in the metabolism of cannabis smoke.

Metabolism studies of Δ^9-THC indicate that it is extensively metabolized by microsomal enzymes in the liver. The metabolism of Δ^9-THC involves oxidation, decarboxylation and conjugation reactions (Chiang and Rapaka 1987). Phase I reactions include allelic oxidation at the C-8 and C-9 positions to yield 11-OH-THC. Other metabolites of Δ^9-THC identified in plasma and feces after intravenous exposure to Δ^9-THC are the alpha and beta isomers of 8-OH-Δ^9-THC (Wall and Perez-Reyes 1981). 11-OH-THC is further metabolized to 8-11-dihydroxy-THC, and then to THC-COOH. More than 100 Δ^9-THC metabolites, including di- and tri- hydroxy compounds, ketones, aldehydes and carboxylic acids have been identified. THC-COOH and the glucuronic acid conjugate are the major end products of biotransformation in most species, including humans (Chaiffetz et al. 2011; Desrosiers et al. 2014a; Huestis 2007; Wu et al. 2018).

Exposure to cannabis smoke has been shown to induce some of the same enzymes that are involved in its metabolism, such as cytochrome P450 1A1. Rat liver levels of P450 1A1 were increased up to two times that of basal levels following cannabis smoke exposure (Marcotte et al. 1975).

Excretion

The elimination of Δ^9-THC and its metabolites occurs via the feces and urine, and to a lesser extent, through sweat, saliva (Huestis 2005; McGilveray 2005), breast milk (Grotenhermen 2003), and hair (Huestis 2005; Musshoff and Madea 2006).

Urine

In one study in humans, 10 – 30 % of an administered dose of radiolabeled Δ^9-THC was excreted in urine during the first day and excretion continued over one week after either i.v., inhalation or oral exposure. Only small amounts of the parent compound Δ^9-THC and the metabolite11-OH-THC were detected in the urine, while approximately 80% of

the radiolabel excreted in the urine was associated with uncharacterized polar metabolites (Lemberger 1972). It was later established that the principal urinary Δ^9-THC metabolite is THC-COOH, which is excreted mainly as a glucuronic acid conjugate (Giroud et al. 2000; Lemberger et al. 1970; Lemberger et al. 1972; Musshoff and Madea 2006; Wall and Perez-Reyes 1981).

Urinary elimination was studied in volunteer patients (13-18 years old) admitted to a drug recovery program. Urine samples were collected at admission (time 0) and every second day until two negative samples were obtained. The elimination time for THC-COOH ranged from 8 – 28 days, and displayed an initial, pronounced decline over the first three to six days, followed by a slower decline (Beardsley and Christensen 2007; Huestis et al. 1996; Huestis and Cone 1998; Wasserman et al. 2015).

Feces

In humans, up to 5 – 10 % of an oral dose of Δ^9-THC was excreted unchanged in feces (Lemberger et al. 1972; Wall and Perez-Reyes 1981), and likely represents the portion of the administered oral dose that was not absorbed, since Δ^9-THC was not identified in feces after i.v. exposure (Lemberger et al. 1970; Lemberger 1972). In these i.v. and oral exposure studies, about 45 – 50 % of the Δ^9-THC metabolites were recovered in feces, with only a small proportion identified as 11-OH-THC and the majority characterized as a more polar metabolite (probably THC-COOH which had not been identified as a Δ^9-THC metabolite at the time) (Lemberger et al. 1970; Lemberger 1972).

Saliva

Δ^9-THC is also excreted through saliva. Peak levels of Δ^9-THC are measured in oral fluid within 30 minutes of smoking (Fabritius et al. 2013).

Clearance

In humans after i.v. exposure, a two phase plasma clearance for Δ^9-THC was observed, with an initial rapid decline in plasma concentration over the first hour and then a much slower decline in plasma concentration lasting for up to 60 h (Lemberger et al. 1970). Wider ranges for the plasma Δ^9-THC half-life have been reported in other human studies:

- 150 min during an 8 h observation period after smoking cannabis containing approximately 60 mg Δ^9-THC (Hunault et al. 2010)
- 1.15 days (during the initial phase) and 8.22 days for the terminal half-life in 13-18 year olds in a drug recovery program (Beardsley and Christensen 2007)
- 50-60 h to 5-6 days in other studies (Huestis 2007; Wall and Perez-Reyes 1981).

Plasma clearance of Δ^9-THC was reported in a cannabis smoking abstinence study of 28 male chronic cannabis smokers with smoking histories that ranged from 4 to 24

years. Plasma Δ^9-THC concentrations decreased during the first 24 h of smoking abstinence, and remained detectable for up to 48 h for the majority of participants; with 5 participants having detectable Δ^9-THC and THC-COOH on day 30 (median Δ^9-THC plasma concentration 0.3 µg/L) (Beardsley and Christensen 2007; Karschner et al. 2016). The number of years of prior cannabis use significantly correlated with Δ^9-THC concentrations on admission, and on days 7 and 14 of the study (Karschner et al. 2016).

The human half-life for THC-COOH has been reported to be in the range of 25 – 35 days (Huestis et al. 1996; Huestis and Cone 1998).

In rats, a plasma Δ^9-THC half-life of 3.7 h was estimated after passive smoke exposure. Clearance was calculated to be 1.1 (L/h) (Ravula et al. 2018).

C. The Endocannabinoid (EC) System

Overview of the EC System

The endocannabinoid (EC) system is comprised of three different plasma membrane receptors: cannabinoid receptor (CBR) 1, 2, and 3, denoted by CB_1R, CB_2R, and CB_3R, respectively. The CB_3R is also known as GPR55 and is not as well studied as the other two receptors.

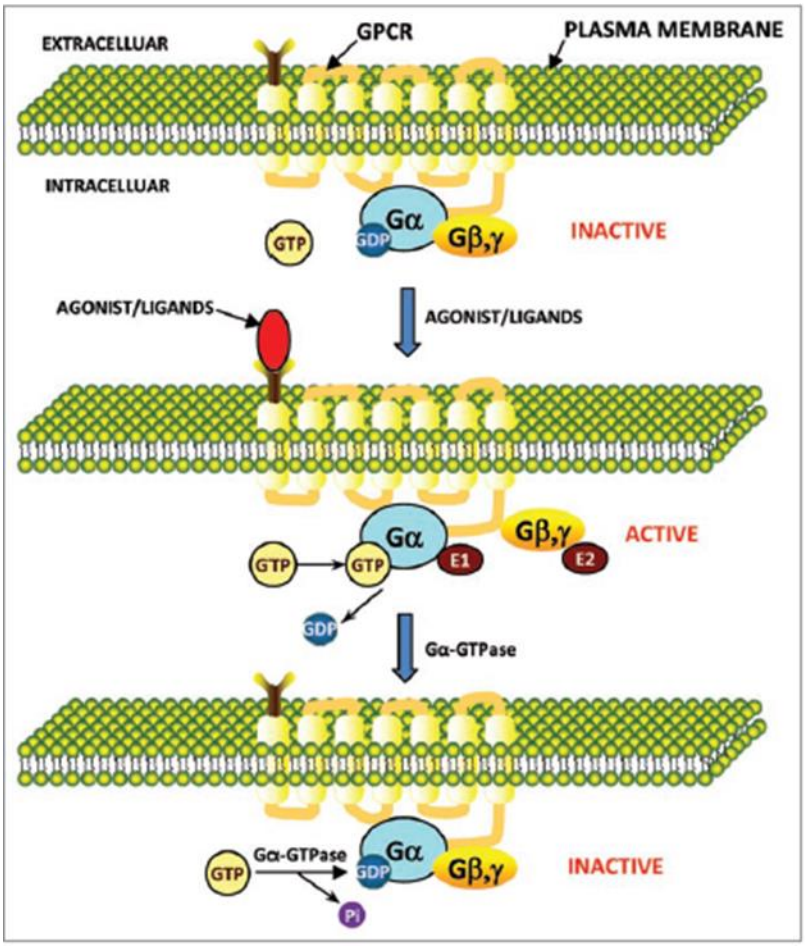
CBRs have different endogenous ligands, but the two most thoroughly studied endogenous cannabinoids (eCBs) are *N*-arachidonylethanolamide (anandamide, AEA) and 2-arachidonylglycerol (2-AG) (Cassano et al. 2017; Keimpema et al. 2011). These ligands are lipophilic derivatives of arachidonic acid (Alpar et al. 2016) and are synthesized "on demand" from membrane phospholipids, depending on physiological need, and bind to the CBRs with different affinities. AEA is a partial agonist of CB_1Rs and CB_2Rs receptors and 2-AG is a full agonist of both these receptors. Upon transport into the cell, AEA and 2-AG are degraded via either hydrolysis or oxidation. AEA can be hydrolyzed by fatty acid amide hydrolase (FAAH) or oxidized by cyclooxygenase (COX), while 2-AG can be hydrolyzed by monoacyl-glycerol lipase (MAGL) or oxidized by lipoxygenase (LOX) (Kano et al. 2009). Exogenous ligands, including Δ^9-THC, are not broken down by the same enzymes that degrade AEA and 2-AG, e.g., FAAH and MAGL. Thus, exposure to exogenous cannabinoids such as Δ^9-THC not only adds to the baseline level of CBR activation due to the action of endogenous ligands, but is also likely to result in a more prolonged activation of CBRs.

Exogenous ligands of CBRs include the cannabinoids present in plants in the genus *Cannabis*. *Cannabis sativa* contains at least 60 different cannabinoid compounds (Dewey 1986). These exogenous cannabinoids include Δ^9-THC, Δ^8-THC, and cannabinol (Atakan 2012). In addition, a number of research compounds have been synthesized that are CBR ligands. These include the CBR agonist HU-210, which is structurally similar to Δ^9-THC, as well as a number of other ligands that are structurally distinct from Δ^9-THC, such as the CBR agonists WIN 55,212-2 and CP55,940 (Wiley et al. 2011).

CBRs are part of the superfamily of G-protein coupled receptors (GPCRs) with seven transmembrane helices (Turu and Hunyady 2010) that interact with guanosine triphosphate (GTP) binding regulatory proteins called G proteins. G-proteins are composed of three subunits: Gα (Gα subunits include four subgroups: Gαs, Gαi/o), Gβ, and Gɣ. When GPCRs are activated, GTP binds to the Gα subunit, inducing a conformational change such that the Gα subunit dissociates from the Gβγ subunits, and activates downstream effectors. This signaling is terminated by the GTPase domain of the Gα subunit, where GTP is hydrolyzed to guanosine diphosphate (GDP) (Wu et al.

2010a; Wu et al. 2010b). It is important to note that the CBRs exist in different activated states capable of coupling to different G proteins. Binding of a specific agonist stabilizes a distinctive active state that can favor coupling to a specific G protein (Lauckner et al. 2005). **Figure 1** below, reproduced from Tuteja (2009) (Tuteja 2009), depicts this process.

Figure 1. Model for signal transduction by activation/inactivation of heterotrimeric G proteins through GPCR. (Source: Tuteja 2009)



The subunits of heterotrimeric G proteins (Gα and Gβγ) in their inactivated state are associated with each other. In inactivation state the GDP is bound to Gα (Gα-GDP). In signal transduction, first the GPCR gets activated by changing its conformation which resulted from binding of agonist/ligands to the extracellular region of GPCR. This activated GPCR further activate the inactive G protein to active G protein complex by dissociating the Gα from Gβγ. In active state the GTP is bound to Gα (Gα-GTP). Now free Gα and Gβγ have their own effectors (E1 and E2, respectively) to further transmit the signals and initiate unique intracellular signaling responses. Later, after the signal transduction, the Gα-GTPase activity hydrolyze the bound GTP (Gα-GTP) to GDP and Pi and inactivate the G protein complex by re-associating the Gα with Gβγ. In this state again GDP is bound to Gα (Gα-GDP) in the G protein complex. In this way the activation and inactivation cycle is completed.

Exogenous or endogenous ligand binding to CBRs causes downstream activation and/or inhibition of different signaling cascades including, but not limited to, PLC (phospholipase C), Akt/PKB (protein kinase B, AC, RhoA (Ras homolog gene family, member A), and JNK (c-Jun N-terminal kinase) cascades.

The EC system has many physiological roles including, but not limited to, maintenance of various stages of pregnancy (Taylor et al. 2010), reproductive function (Correa et al. 2016; Maccarrone et al. 2015a), somatic development (e.g., bone growth and differentiation) (Wu et al. 2010a), regulation of the immune system, apoptosis (Rieder et al. 2010), and neurodevelopment (Keimpema et al. 2011). The CBRs are expressed throughout the body:

> "CB1 receptors are expressed in the central nervous system (CNS) and are particularly rich in certain brain areas such as basal ganglia, cerebellum, and hippocampus. CB1 receptors are also found in the periphery, including human testis, sperm cells, embryos, retina, colonic tissues, peripheral neurons, adipocytes, and other organs including human adrenal gland, heart, lung, prostate, uterus, oviduct, and ovary" (Reggio 2010).

CB_2R is mainly present in peripheral tissues and also in the nervous system. These peripheral tissues include: cardiovascular system, liver, gastrointestinal tract, B and T lymphocytes, osteoblasts, developing embryos, embryonic stem cells, human uterus and myometrium, mouse Sertoli cells, epidermis and sebaceous glands (Maccarrone 2008; Maccarrone et al. 2015a). The function of the CB_3R (GPR55) is not well understood, however, it is expressed in the nervous system (Basavarajappa et al. 2009).

Some of the signaling pathways of the EC system that can be affected by in utero exposure to Δ^9-THC and other exogenous cannabinoids present in cannabis smoke and vapor are described below. The following sections focus on the role of the EC system in regulating:

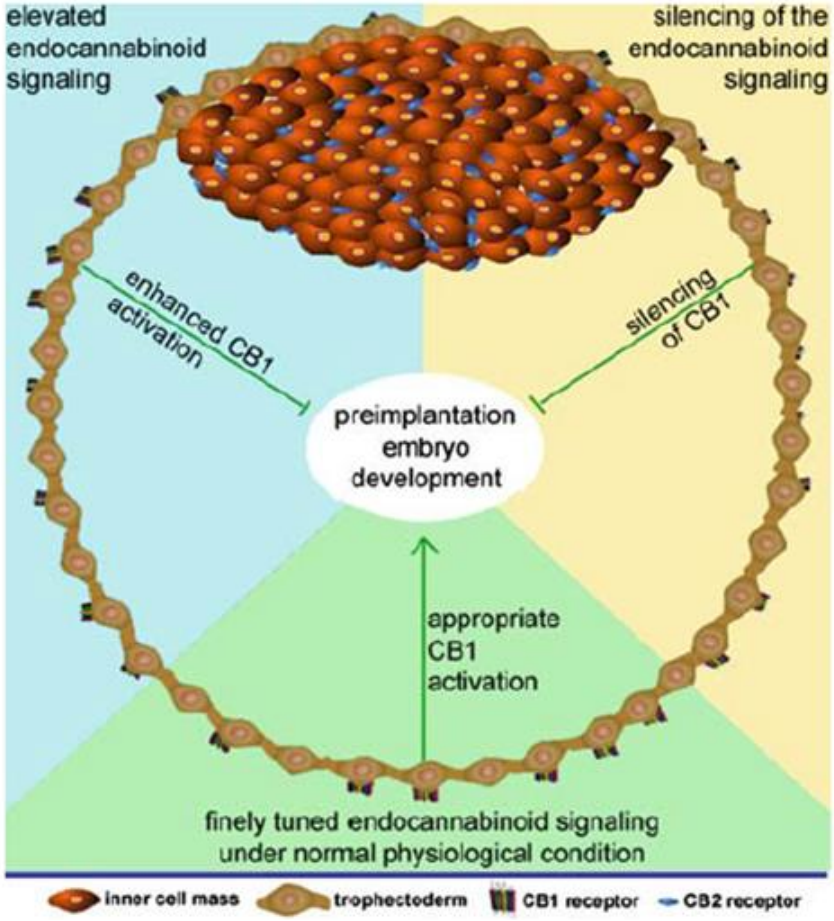
(i) development of the embryo and facilitating successful embryo implantation.
(ii) bone growth and differentiation.
(iii) development of the immune system, and
(iv) development of the nervous system.

Embryo development and implantation

Embryo development proceeds from a fertilized ovum (zygote) to a 2-, 4-, 8-cell embryo, to the 16-cell morula stage, to the blastocyst stage that it is ready for implantation. The developing embryo expresses CB_1R and CB_2R, beginning at the 2-cell stage, and continuing through to the blastocyst stage (Paria et al. 1995; Yang et al. 1996). The

blastocyst consists of two different cell populations, the inner cell mass (ICM) that will develop into the embryo and the surrounding group of cells known as the trophectoderm (TE) that will establish the cellular connections with the mother's uterus to develop the placenta (see Figure 2, reproduced from (Sun and Dey 2009; H Wang et al. 2006a). The ICM expresses CB_2R, and the TE expresses CB_1R.

Figure 2. Overview of cannabinoid signaling in preimplantation embryo development (Source: Sun et al. 2009)



Exaggerated signaling mediated by CB_1R leads to aberrant preimplantation embryo development.

In vitro studies of mouse embryos have shown that CB_1R agonists delay embryo development, inhibiting blastocyst formation, zona hatching and trophoblast outgrowth (Paria et al. 1995; Schmid et al. 1997; Yang et al. 1996). The G-protein-coupled CBRs inhibit adenylyl cyclase (AC) and N-type Ca^{2+} channels (Paria et al. 1995). When a CBR agonist binds to either receptor, the receptor will activate Gαi, which in turn inhibits AC, leading to reduced levels of cAMP and intracellular calcium (Ca^{2+}) levels (Wang et al. 1999). A close balance of cAMP levels and Ca^{2+} is thought to be part of normal embryonic development (Paria et al. 1995).

Therefore, a possible mechanism of action could be that the activation of CB_1R may inhibit N-typeCa^{2+} channels, which may alter intracellular Ca^{2+} levels and interfere with cell polarity and embryonic compaction, thereby delaying transformation from the morula to the blastocyst. Δ^9THC has the ability to inhibit forskolin-stimulated cAMP accumulation (Paria et al. 1995; Yang et al. 1996).

Oviduct transport

The EC system plays a key role in the transport of the embryo through the oviduct to the uterus. Dysfunctional regulation of this signaling system may result in a deficient embryo transport to the uterus, with the end result of an ectopic pregnancy (Maccarrone 2008; Sun and Dey 2008).

The embryo transported through the oviduct into the uterus is controlled by various factors such as steroid hormone balance and the sympathetic nervous system through modulation of α_1-adrenoceptors (α_1-AR) and β_2-adrenoceptors (β_2-AR) in the oviduct. Stimulation of α_1-AR results in muscle contraction, whereas stimulation of β_2-AR stimulates muscle relaxation. Continuous activation and relaxation of the oviduct muscle creates the means to transport the embryo into the uterus. CB_1Rs are co-localized in the oviduct with α_1-ARs and β_2-ARs. The co-localization of CB_1R with β_2-AR and a direct physical interaction of these two receptors has been demonstrated by Hudson et al. (2010). Oviducts in CB_1R null mice show increased levels of norepinephrine (NE) and CB_1R-mediated signaling is coupled to adrenergic signaling (demonstrated by Hudson et al. 2010). This suggests that exposure to CBR agonists can produce a cross-desensitization of β_2-ARs (Hudson et al. 2010), with possible consequent effects on the regulation of the peristalstic (contraction and relaxation) action of the oviduct. Exogenous cannabinoids have been shown to relax the smooth muscle of the mouse oviduct, and prevent the transport of the embryo into the uterus for implantation (Sun and Dey 2009).

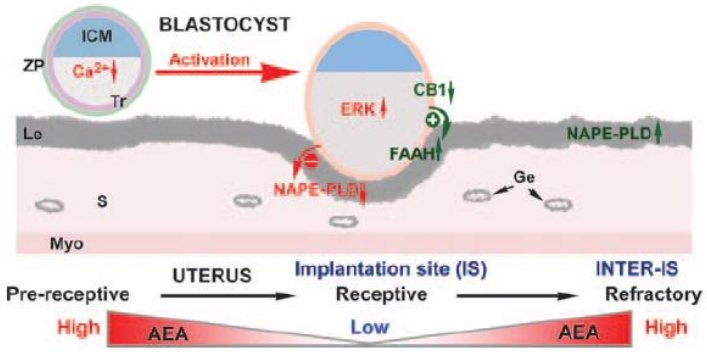
Implantation

At the blastocyst stage, the TE cells of the embryo contact the uterine endometrium to begin the process of implantation. Successful implantation requires an interaction between the blastocyst and the uterus when the uterus is in its “receptive” state. Activation of CB_1R (via AEA) and AEA hydrolase in the uterine epithelium is enhanced by a “FAAH activator” released by the embryo itself that modulates the levels of AEA (Maccarrone 2009). Normal development of the embryo plays a key role in inducing the necessary changes in the uterus to create the receptive uterine environment needed for successful implantation, as shown in Figure 3. This involves activation of the ERK and Ca^{2+} signaling pathways. As stated by Wang et al. (2006b),

> “AEA at a low concentration activates ERK signaling in dormant blastocysts via CB1. In contrast, at higher AEA levels, it fails to achieve ERK activation, but

instead inhibits Ca^{2+} mobilization. This finding provided for the first time a potential 'cannabinoid sensor' mechanism to influence crucial steps during early pregnancy. An association of spontaneous pregnancy loss with elevated peripheral AEA levels in women is consistent with the observations in mice. These findings in mice and humans reinforce the concept that endocannabinoid signaling is at least one of the pathways determining the fate of embryo implantation. In this regard, there is evidence that activation of CB1 inhibits human decidualization and promotes apoptosis of decidual cells *in vitro,* thus adding a new role of endocannabinoids in human pregnancy."

Figure 3. Endocannabinoid signaling in blastocyst activation and implantation. (Source: Wang et al. 2006a)



Evidence suggests that regulated levels of endocannabinoids, primarily AEA, in the receptive uterus and CB1 in activated blastocysts, are beneficial for implantation, whereas higher levels are detrimental to this process. This biphasic role of AEA is further supported by findings that AEA within a very narrow range regulates blastocyst activation and implantation by differentially modulating ERK signaling and Ca^{2+} channel activity via CB1. Uterine AEA levels conducive to implantation are primarily regulated by the coordinated expression and activity of *N*-acylphosphatidylethanolamine-hydrolyzing PLD (NAPE-PLD) that generates AEA and by FAAH that degrades AEA in the uterus during early pregnancy. In addition, the implanting blastocyst down-regulates uterine NAPE-PLD expression, but enhances uterine FAAH activity via releasing a putative FAAH activator, thus contributing to rapid turnover of AEA at the implantation site. Ge, Glandular epithelium; IS, implantation site; INTER-IS, interimplantation site; Le, luminal epithelium; Myo, myometrium; S, stroma; Tr, trophectoderm" (Taken directly from Wang et al. (2006a))

Immediately after implantation, the trophoblast begins to form and is essential in the proper development of the placenta. Studies in mice have shown that CBR agonists, including Δ^9-THC, can interfere with these processes. For example, low levels of cannabinoid agonists have been shown to accelerate trophoblast differentiation and higher levels inhibit trophoblast proliferation and gene transcription, and these effects are mediated through CB_1R (Maccarrone et al. 2002; Paria et al. 1998; Paria and Dey 2000; Wang et al. 1999; Yang et al. 1996). Trophoblasts secrete proteases that allow

invasion of its cells. The invasion of the trophoblast cells into the endometrium to establish the fetal blood flow supply is regulated by many signaling pathways, such as cytokines (leukemia inhibitory factor (LIF), interleukin-6 (IL-6), and granulocyte macrophage colony stimulating factor. These cytokines and stimulating factors activate various intracellular signaling pathways in trophoblast cells, one of which is the signal transducer and activator of transcription 3 (STAT3) which is part of the Janus kinase signal transducers (JAKs-STAT). The activation of STAT3 by phosphorylation allows regulation of cell motility, a key step in trophoblast cell invasion. After cytokine binding to the surface of the receptor, JAKs cross-phosphorylate and activate each other allowing intracellular STATs to bind to the receptor domains on the cytoplasmic domain of the cytokine receptors. After activation, STATs dissociate from the receptor and translocate into the nucleus of the cell and can up-regulate the transcription of target proteins by binding to their promoter regions. One such protein is the SOCS protein that negatively controls the duration of cytokine signaling (Fitzgerald et al. 2008).

CB_1R ligands, including Δ^9-THC, cause trophoblast dysfunction by suppressing STAT3 signaling and ultimately disrupting placental development by impairing the migration and invasion of trophoblasts. In mice exposed to Δ^9-THC, there was a significant decrease in phosphorylation of STAT3 in the placenta, which was consistent with the *in vitro* results (Chang et al. 2017). Phosphorylation of STAT3 is essential in regulating cell motility, which is a critical step in trophoblast cell invasion.

Pretreatment of trophoblast cells *in vitro* with CBR antagonists reversed the effect of Δ^9-THC regarding the migration of cells and confirmed that this effect is mediated via CBRs. The effect of Δ^9-THC on cell migration and invasion was seen both *in vivo* (in prenatally exposed mice) and *in vitro* (Chang et al. 2017). This ultimately results in inadequate nutrition and blood supply to the fetus due to dysfunctional trophoblast migration and improper placental development.

Immune System Development

The immune system is comprised of an innate and an adaptive system. The innate immune system reacts in a non-specific manner to invasive pathogens and tumor cells, and is comprised of macrophages, dendritic cells, neutrophils, natural killer cells, eosinophils, basophils, and mast cells. The adaptive immune system reacts to non-host antigens, e.g., pathogens and tumor cells, in an antigen-specific manner and retains antigen-specific memory cells. It is comprised of dendritic cells, B lymphocytes, and T lymphocytes (Chiurchiu et al. 2015; Maccarrone et al. 2015b; Rieder et al. 2010; Wolfson et al. 2016).

The EC system plays a significant role in the function of the immune system. CBRs areexpressed on several types of immune cells, including cells involved in innate, and

cells involved in adaptive immunity. Fetal immune cells have also been shown to express CBRs. In fact, the EC system plays an important role in the immune system during pregnancy through the adaptive immune system. During pregnancy the maternal adaptive immune system undergoes changes, including suppression of the "non-host" response, to permit toleration of the semiallogeneic fetus. Expression of CB_1 and CB_2 receptors is differentially regulated in B cells during pregnancy (Wolfson et al. 2016), which affects cytokine production by B cells.

Most types of immune cells express both CB_1R and CB_2R, and some, such as Natural Killer (NK) cells, also express CB_3R. In general, the level of CBR expression varies by immune cell type in the following rank order, from highest expression to lowest: B cells > NK cells > monocytes/ macrophages > neutrophils > CD8 T cells > CD4 T cells (Rieder et al. 2010).

In the innate immune system, CB_1Rs and CB_2Rs are highly expressed in macrophages (Chiurchiu et al. 2015). Dendritic cells express both CB_1Rs and CB_2Rs and through activation of these receptors, they function to suppress the immune system (Chiurchiu et al. 2015).

The EC system is heavily involved in adaptive immunity as well, through activation of various signaling cascades. CB_2R activation has been shown to suppresse the immune system via a Ca^{2+} dependent mechanism (increased intracellular Ca^{2+} levels). There is also some evidence suggesting that eCBs can modulate immune function through CBR-independent mechanisms (Maccarrone et al. 2015b). In addition, activation of CBRs can lead to apoptosis, which may have implications for development of the fetal immune system (Dong et al. 2019). Furthermore, exogenous cannabinoids (such as Δ^9-THC) may alter immune function by induce epigenetic changes, such as altered DNA methylation and histone acetylation in T1 and T2 helper (Th_2 and Th_1) associated genes (Dong et al. 2019).

Bone Growth

Bone growth is a continuous process that begins prenatally and ends in maturity when the epiphyseal plates are fully ossified (Cooper et al. 2013; DeSesso and Scialli 2018). Bone growth is the result of a delicate balance between osteoblasts (bone forming cells) and osteoclasts (bone resorbing cells) activity. It starts with osteoclast precursors being recruited to bone, followed by maturation of these cells to form resorptive osteoclasts. These osteoclasts then generate resorptive lacunae (pits), into which new bone, produced by the osteoblast, is deposited. An imbalance between bone formation and resorption can manifest as either bone mass accrual, or bone loss (Bab and Zimmer 2008; Wu et al. 2010b).

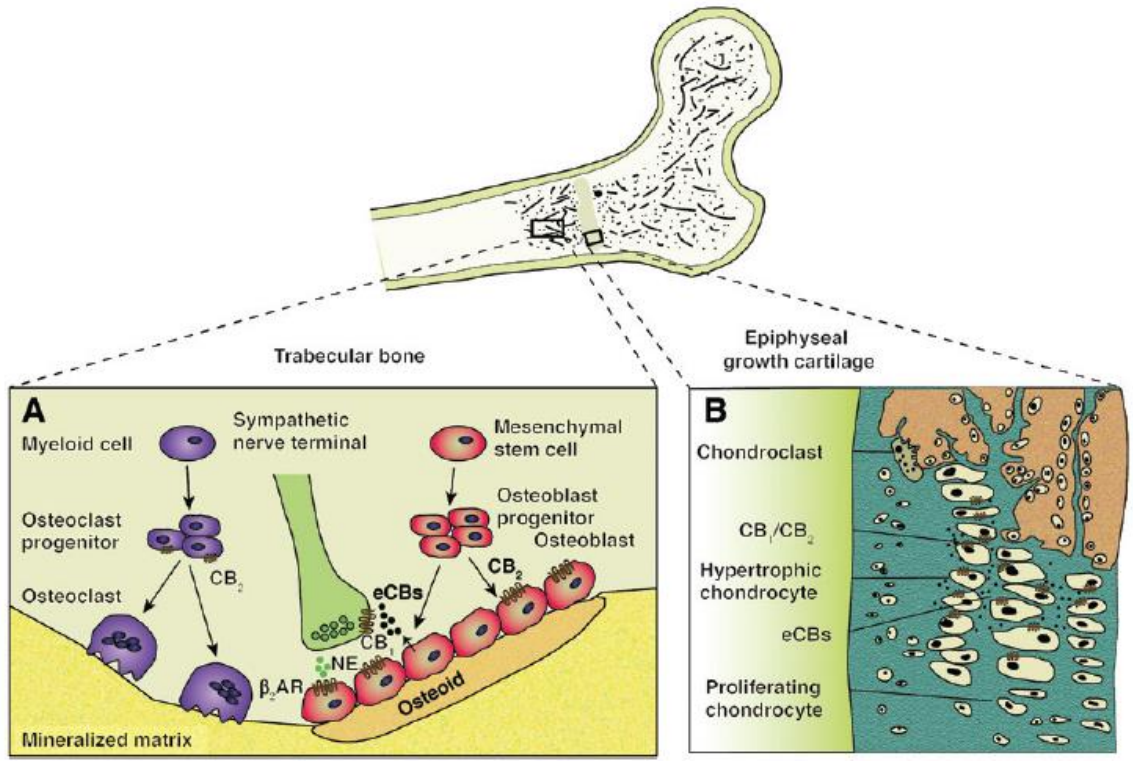
The EC system plays a significant role in bone growth and differentiation. The signaling pathways and molecular mechanisms of the EC system in bone are very similar to that in other tissues.

Receptors: CB_1Rs are expressed in sympathetic nerve endings proximal to the bone (See

Figure 4). The osteoblast cells express β2-adrenergic receptors (β_2AR). CB_2R are expressed in osteoblasts, osteocytes, and osteoclasts and regulate bone mass by negative modulation (Ofek et al. 2006).

Ligands: eCBs produced by the osteoblast would bind the CB_1Rs in the nerve terminal, activation of these receptors would down-regulate noradrenaline (NE) production which is the natural ligand for β_2AR. The consequence of this is a reduction on the negative control that NE has on osteoblast activity (Bab and Zimmer 2008). In bone and bone cell cultures 2-AG activates CB_1R in the sympathetic nerve terminals, whereas AEA affects bone cells directly by binding to CB_2R (Bab and Zimmer 2008).

Figure 4. Endocannabinoid Signaling in Bone Growth and Remodeling (Source: Zimmer 2016).



"(A) Bone is richly innervated by sympathetic nerve terminals, which release norepinephrine (NE) and inhibit bone formation by activating β2-adrenergic receptors (β2AR) on osteoblasts. CB1 receptors are present on these nerve terminals and are activated by endocannabinoids (eCBs) released from apposed osteoblasts. CB1 signalling inhibits NE release, thus reducing the sympathetic tone and alleviating its inhibitory effects. CB2 receptors are mostly present on osteoblasts and osteoclasts. Activation of CB2 receptors enhances the proliferation of osteoblast progenitors and restrains osteoclastogenesis. Although bone remodeling is stimulated in CB2-deficient mice, there is a net loss of bone mass, which results in an age-related osteoporosis phenotype. (B) Cannabinoid CB1 and CB2 receptors, as well as endocannabinoid synthetic enzymes, are also expressed in the epiphyseal growth cartilage. CB1 is mostly present in the hypertrophic cell layer. CB2, DAGLα and DAGLβ are found in the transitional zone between proliferating and hypertrophic cells. The epiphyseal growth cartilage contains significant levels of 2-AG. Mice lacking CB2 receptors have longer femora and vertebral bodies resulting in a longer stature, whereas stimulation of CB1 restrains bone growth." (Zimmer 2016)

As shown schematically in

Figure 4 above, CB_1Rs and CB_2Rs are expressed in epiphyseal growth cartilage (EGC) hypertrophic chondrocytes. 2-AG, and two enzymes involved in 2-AG synthesis, diacylglycerol lipase-alpha (DAGLα) and -beta (DAGLβ), are also expressed in EGC hypertrophic chondrocytes. CB_1Rs and CB_2Rs as well as enzymes involved in the synthesis of eCBs are expressed on hypertrophic chondroblasts in the epiphyseal growth cartilage plate. CB_2R signaling stimulates proliferation of osteoblast progenitors and affects differentiation of osteoclasts. CB_1Rs are most prominently expressed on sympathetic nerve terminals and inhibit release of norepinephrine, thus reducing the sympathetic tone which in turn inhibits bone formation (Maccarrone et al. 2015b).

The expression of CB_2Rs in bone cells means that eCBs could exert their signaling effects through a cell-autonomous way. Exogenous cannabinoids may affect these signaling pathways and thus may affect bone growth and/or differentiation. *In vivo* and *ex vivo* studies have shown effects of Δ^9-THC on skeletal elongation and on epiphyseal growth cartilage chondrocyte activity (Wasserman et al. 2015).

Neurodevelopment

Involvement of the EC system during early brain development has been recognized for some time (Fernandez-Ruiz et al 1999; 2000), and while recognition of the importance of proper endocannabinoid signaling in neurodevelopment has prompted much reseach in this area (Calvigioni et al 2014), current understanding of the mechanisms through which neurodevelopment and neurobehavior are shaped by these signaling pathways remains limited.

CBRs are expressed in various parts of the central nervous system (CNS) in both the brain and the spinal cord. These areas include expression in the (Kano et al. 2009):

- innermost layers of the olfactory bulb, hippocampus (high levels in the dentate molecular layer and the CA3 region), lateral part of the striatum, target nuclei of the striatum (globus pallidus, entopeduncular nucleus, substantia nigra pars reticulata), and cerebellar molecular layer;
- forebrain regions, where moderate levels are expressed;
- cerebral cortex (higher in the frontal, parietal, and cingulated areas than other cortical areas), septum, amygdala (nucleus of lateral olfactory tract), hypothalamus (ventromedial hypothalamus), lateral subnucleus of interpeduncular nucleus, parabrachial nucleus, nucleus of solitary tract (caudal and commissural portions), and spinal dorsal horn;
- thalamus, and spinal ventral horn(Kano et al. 2009).

The associated subunits of CBRs direct signaling through many cascades, such as via inward rectifier K^+ (GIRK) channels, modulation of adenylyl cyclase, cyclic adenosine monophosphate (cAMP) dependent protein kinase (PKA) and most importantly release

of calcium (Ca^{2+}) from intracellular stores where Ca^{2+} can then directly bind to and regulate ion channels (Bloom 2006a).

Cannabinoid receptors (CB_1R, CB_2R and CB_3R) are transiently distributed during the development of the nervous system, suggesting that these receptors are involved in the overall regulation of structural and functional brain maturation (Berrendero et al. 1998; Keimpema et al. 2011).

Ligand binding to CBRs within the developing brain can result in downstream activation and/or inhibition of different signaling cascades, affecting key aspects of CNS development, such as neurite outgrowth.

> "Activation of CB_1Rs in developing neurons leads to neurite outgrowth and affects growth cone steering decisions. The unique configuration of CB_1Rs and 2-AG synthesis enzymes in the growth cone allows for a primarily autocrine activation of CB_1Rs, as well as the paracrine signaling amongst neighboring neuronal segments or growth cones advancing in parallel and coalescing into axonal bundles. To prevent ectopic activation of CB_1Rs, either expressed or transported along the axon, and consequently, unwanted neurite outgrowth or steering decisions, MAGL is expressed in the stabilized neurite segment to scavenge excess 2-AG. When the growth cone reaches its postsynaptic target, the premature presynapse halts by adopting the "adult configuration" of 2-AG signaling by the redistribution of DAGLs and MAGL" (Calvigioni et al. 2014).
>
> "MAGL, or any other endocannabinoid-related catabolic enzyme known to date, is not able to degrade THC. Thus, introduction of THC to developing foetal circuits could result in the ectopic activation of CB_1Rs, leading to unwanted directional neurite outgrowth, including synapse formation errors. In addition, THC exposure increases the synthesis and release of endocannabinoids, particularly AEA, in a concentration-dependent manner through CB_1Rs... In response to chronic THC or synthetic cannabinoid agonist exposure, CB_1Rs undergo downregulation and rapid desensitization in a regionally distinct manner with considerable magnitude. In sum, besides out-of-place activation of CB_1Rs, THC is able to reshape endocannabinoid signaling by directly affecting receptor and enzyme levels. The tightly regulated spatiotemporal expression of endocannabinoid-related enzymes and receptors during foetal development thus predicts sensitivity to prenatal cannabis exposure" (Calvigioni et al. 2014).

The role of the EC system in neurodevelopment is discussed in more detail in **Appendix 4.** Animal Neurodevelopmental Toxicity Studies)

D. Human Studies of Developmental Effects

The initiation of the epidemiological review begain with a systematic search of the scientific literature for studies that could best inform the evaluation of the effects of prenatal cannabis exposure on human development.

Tabulation and Summarization of Epidemiologic Studies

Study selection for detailed summaries

Detailed summaries were developed and included in the Appendices for analytic epidemiological studies with individual exposure and outcome assessment, such as cohort and case-control studies, that met the criteria for inclusion discussed above. Ecologic studies, cross-sectional studies, and case series were excluded. Studies that did not address potential confounding by tobacco, alcohol, and other drugs were also excluded from tabulation with few exceptions where this was noted in the detailed appendix tables or text. Included studies reported original data analyses with details about methods, and were published in peer-reviewed journals. Abstracts from conferences, opinions, and reviews were excluded.

In the human studies many publications used different terms in reference to cannabis including marijuana. For ease of reading the term cannabis is used in this document to represent marijuana. Other forms are included as they were presented in the original study.

Summary tables

To facilitate consideration of this large dataset, the tables and figures for the human studies of developmental outcomes are presented in order of increasing detail. Thus, Table D.4 is a list of the studies of birth outcomes, organized chronologically, which provides a high level overview of the scope of the dataset. Table D.5 provides more detailed information of each study concerning study design and exposure, organized by cohort, where applicable, and chronologically. This table, however, is still intended as an overall reference for the dataset.

SimilarlyTable D.12 is a list of the studies of neurodevelopmental outcomes, organized by age/developmental stage, and chronically, which provides a high level overview of the scope of the dataset. Table D.13 provides more detailed information of each study concerning study design and exposure, age/developmental stage, where applicable, and chronologically.

Detailed study summaries

In Appendix 1 and 2, detailed summaries are provided for each study of birth outcomes and neurodevelopment. These studies are organized in two tables; Table 1.1 includes

studies of birth outcomes, ordered alphabetically, while Table 2.1 includes studies of neurodevelopmental outcomes, presented by outcome, ordered chronologically. A small number of studies that are less informative than the tabulated studies have been summarized in narrative form and are presented following the Appendix tables.

The epidemiologic studies summarized in these tables include information relevant for assessing the quality of each study and its ability to provide evidence on whether or not prenatal exposure to cannabis or Δ^9-THC can cause adverse effects in offspring. Each column includes information needed to identify each study and assess its ability to address the HID's research question.

In addition to these detailed summaries the Developmental and Reproductive Toxicant Identification is being provided the original publications on all the summarized studies.

Information for the Detailed Summary Tables

Study design/sample [sizes] includes the study design (retrospective or prospective cohort, case-control, other), a brief description of the population sample, number of subjects, and the years of exposure data collection, if reported. The sample size is presented using the abbreviation N for the initial study population and n for the resulting sample population after any exclusion or loss to follow-up, etc. Statistical analysis methods are also noted here.

Outcomes of Interest includes developmental outcomes the authors evaluated, such as birth weight, gestation length, and behavior problems.

Unless otherwise noted: low birth weight was defined as birth weight less than 2,500 grams; small for gestational age was the lowest 10^{th} percentile of birth weight for each gestational week, and preterm birth was <37 weeks gestation.

Exposure Considerations

Exposure assessment

Prenatal exposure to cannabis in the studies reviewed in this document was predominantly assessed through maternal self-report during prenatal interviews. Biological analysis using urine samples, and less frequently meconium, were also used in some studies. Agreement between the results from self-reported use and biological analysis varied across studies that assessed both measures. Studies have shown women who report cannabis use do not always test positive in urine samples. This may be due to a number of factors including the limit of detection of the tests methods and the half-life of Δ^9-THC in the body. Conversely, some women who report not using cannabis do have a positive urine test. This most likely is due to under-reporting of use. Although there may be exposure to secondhand cannabis smoke in high enough concentrations to result in positive urine tests (Cone et al. 2015), the ambient air

concentrations need to be high. For exposure assessment through meconium samples, disagreement between self-report and meconium analyses may be to some degree a matter of the difference in the time frame for the self-reported use versus the time frame of exposure reflected in the meconium sample, primarily later in pregnancy.

In a comprehensive review of the literature, Chiandetti et al. (2017) examined the comparison between self-reported consumption of cannabis and biomarkers of exposure. The percentage of women who self-reported exposure varied from 0 to 2.9%, while positive biomarker results ranged from 4 to 12.4%. These results suggest that self-reported exposure has the potential for substantial underestimation of prenatal cannabis exposure.

Imprecise exposure assessment resulting in non-differential misclassification of exposure would likely bias the estimate of any association of risk towards the null, that is, toward not detecting an effect even if one were present.

Changes in cannabis potency over time

An important factor to consider in assessing the overall findings of the studies is the well-documented increase in the potency of cannabis over time. There has been a large increase in potency of cannabis over the last few decades.

Elsohly et al. (2000) analyzed 35,312 samples of cannabis preparations confiscated by the US Drug Enforcement Administration from 1980-1997. The results showed

> "the potency (concentration of Δ^9-THC) of marijuana samples rose from less than 1.5% in 1980 to approximately 3.3% in 1983 and 1984, then fluctuated around 3% till 1992. Since 1992, the potency of confiscated marijuana samples has continuously risen, going from 3.1% in 1992 to 4.2% in 1997."

Further work by Elsohly et al. (2000) found the increase in potency of confiscated cannabis plant material from ~4% in 1995 to ~12% in 2014, "resulting in a change in the ratio of Δ^9-THC to cannabidiol from 14 times in 1995 to ~80 times in 2014" (ElSohly et al. 2016).

Many of the studies presented in the document were conducted beginning in the late 1970's, or early 1980's when cannabis was much less potent. One consideration in evaluating consistency of studies of comparable quality and power is the increasing potency in cannabis, and exposure to higher amounts of THC, over time.

Longitudinal Cohort Studies

Ottawa Prenatal Prospective Study (OPPS)

Recruitment

The Ottawa Prenatal Prospective Study (OPPS) is a longitudinal cohort that evaluated prenatal lifestyle habits and their effects on the offspring (Fried 1980). The studies from this cohort are shown in the table below. The study recruited pregnant women through notices in public media or in waiting rooms of prenatal clinics in the four largest Ottawa hospitals (Fried 1985). Recruitment started in 1978-1985 (Fried 1989). The study was described as an investigation of prenatal influences on the developing fetus, with the investigators being interested in all women, regardless of prenatal habits (Fried et al., 1980). Mothers who were interested in participating were instructed to call the researchers by phone or to mail a pre-addressed, stamped post card available from the receptionist or affixed to the notices (Fried et al., 1980). The demographics of the women are described as “middle class, urban, and low-risk population” (Fried 1989). Women who reported use of opiates, amphetamine, and cocaine more than six times during the year before pregnancy or more than once during pregnancy were not included in the study (Fried 1980).

Interview methods and information collected

Subjects who volunteered to participate were interviewed once during each remaining trimester. Women who entered the study in the second or third trimester were interviewed “as soon as the contact was made.” Subsequent interviews took place in the sixth and nine months of pregnancy (Fried et al., 1980).

Pregnant women were interviewed by the same female interviewer throughout pregnancy in order to build rapport. The interviews were usually conducted in the home of the participant and collected information on socioeconomic status, mother’s age and health (prior to and during pregnancy), obstetrical history of previous pregnancies, father’s medical history, 24-hour dietary recall (including caffeine consumption), and past and present drug use with emphasis on alcohol, cigarettes, and marijuana. Average daily caffeine use was calculated from the 24-hour dietary recall according to the number, size, and servings of coffee, tea, sodas, and other dietary sources of caffeine consumption during pregnancy (Fried and O’Connell 1987).

Data collected at birth included gestation length, type of birth, birth weight, and medication used during labor. The interviews collected information including alcohol, nicotine, and cannabis use the year before pregnancy and during each trimester. If inconsistent information was reported regarding quantity of substance use, the higher quantity was used in data analysis.

The same questions repeated each trimester so that they had a measure of test-retest reliability. The consistency of self-report for cannabis use was very high with fewer than 7% of women inconsistently reporting cannabis usage. If there was inconsistency then the higher number was used in data analysis (Fried 1985).

Sample Size and Follow-up

Sample sizes analyzed in each OPPS study may vary depending on follow up information available. Generally, information was collected from approximately 700 women in the Ottawa area. Approximately 180 offspring from this sample have been followed up beyond the neonatal period (Fried 1995). About one third of the subjects moved from the Ottawa area causing loss to follow up (Fried 1995).

The women were selected to include all those who used cannabis during pregnancy, were heavier social drinkers, and who smoked cigarettes regularly (Fried and O'Connell 1987). Approximately 50 women who did not use cannabis, drink alcohol, and were non-smokers were chosen as controls.

The average age of women in the sample was 28.9 years. The mean family income was $31,500 compared to $36,000 for the Ottawa metropolitan area. The average education level was higher in the sample, with 64% of women achieving a greater than high school education compared to the 49% of women giving birth in the Ottawa region (Fried and O'Connell 1987).

The authors note that the participants were healthy volunteers, the vast majority of whom were middle-class, married, and well-educated, making this a very low risk cohort. Because the sample was selected on the basis of drug use, the proportion of users and extent of use cannot be considered representative of use by a middle class population (Fried and Makin, 1987, p.5).

Exposure Quantification

Information on quantity of use was collected in number of joints. If participants reported use of hashish the amount was multiplied by five to account for the increased THC concentration (Fried 1985).

Exposure quantification of cannabis (e.g. light, moderate, or heavy use) for analysis varies in each study.

Alcohol consumption was broken down into wine, beer, and liquor, using both quantity and pattern to average ounces of absolute alcohol (AA) per day. Alcohol use was classified in four categories based on average weekly consumption: abstainers, those who drank an average <0.14 oz. AA/day (light), those who drank between 0.14 and 0.85 oz. (moderate), and those who drank >0.85 oz. of AA/day (heavy) (Fried et al., 1980 p. 326). The authors considered 0.85 oz. AA, which would be contained in 1.5 bottles of

Canadian beer, two glasses of wine, or two highballs, “at a risk level” (Fried et al., 1980 p. 326).

For cigarette use, the number of cigarettes smoked per day was multiplied by the nicotine content of the brand specified. The three categories were non-smokers, light smokers (those who averaged less than 16 mg of nicotine/day), and heavy smokers (who averaged at least 16 mg of nicotine/day) (Fried et al., 1980 p. 326).

Prevalence of cannabis use in cohort

Usage of cannabis declined significantly after pregnancy, but during each of the trimesters, percentages remained constant (Fried 1985).

Based on Tables 3 and 5 in Fried et al. (1980), of 217 participants for whom there were data on alcohol and marijuana use in the first trimester, 190 did not use marijuana, 20 smoked ≤1 joint per week, four women smoked two to five joints/week, and three women smoked >five joints/week. Table 6 in Fried et al. (1980) shows that 79.6% of the sample were non-users before pregnancy, and 90.2% were non-users by the third trimester (see below).

Table D.1: Levels of Cannabis Use by Trimester

Marijuana use (joints/wk)	**Pre-pregnancy (%)**	**1st trimester (%)**	**2nd trimester (%)**	**3rd trimester (%)**
Non-user	79.6	87.5	87.1	90.2
Light (≤1)	14.8	(9.3)	8.8	7.3
Moderate (2-5)	2.3	1.9	0.9	0.4
Heavy (>5)	3.2	1.4	1.8	2.0
N (from tables 3, 5)	217	217		190

Marijuana use was not statistically associated with alcohol use, except among the heavy users (Fried et al., 1980 p. 335). All heavy marijuana users smoked cigarettes to some degree (Fried et al., 1980 p. 340). Heavy marijuana use was also associated with lower family income.

Table D.2. Studies from the Ottawa Prenatal Prospective Study (OPPS) on the Effects of Prenatal Exposure to Cannabis.

Reference	**Outcome, Timing**	**N, study n**
Fried 1980	Nervous system abnormalities 60-80 hrs postpartum	N = 291, n=89
Fried 1982	Nervous system abnormalities at 4,9, and 30 days, and 1 yr	n = 420

Reference	Outcome, Timing	N, study n
Fried et al. (1984)	BW, GA	n=583
O'Connell and Fried (1984)	Minor anomalies, FAS-like features	n=636
Tansley et al. (1986)	Transient pattern-evoked visual cortical potential (maturation of visual mechanisms), ~8 yrs	N=~700, n=96
Fried et al. (1987)	Neonatal neurologic status at 9 and 30 days	N=~700, n= 247 at 9 days, n=254 at 30 days
Fried and O'Connell (1987)	Growth at 1 and 2 yrs	N=~700, n=123
Fried and Makin (1987)	BNBAS, 3-6 days	N=~700, n=250
Fried and Watkinson (1988)	At 12 mos: HC, Height, Weight, BSID At 24 mos:BSID, Reynell Development Language Scales (at 12 & 24 mos)	N=~700, n=217 at 12 mos, n= 153 at 24 mos
Fried and Watkinson (1990)	Neurobehavior at 36 and 48 mos	N= 698, n= 130 at 36 mos, n= 123 at 48 mos
O'Connell and Fried (1991)	Neurobehavioral development at 6-9 years	N not explicitly stated, n= 56
Fried et al. (1992a)	Attentional behavior and impulsivity at 6 yrs	N = 698, n=126
Fried et al. (1992b)	Cognitive and receptive language development at 5-6 yrs	N=~700, n 135 at 5 yrs, n= 137 at 6 yrs
Fried et al. (1997)	Reading and language at 9-12 yrs	N=690, n= 131
Fried et al. (1998)	Cognitive and executive function at 9-12 yrs	N= 698, n= 131
Fried et al. (1999)	BW, BL, HC, PI, growth from birth to 12 yrs	n =190
Fried and Watkinson (2000)	Visuperceptual performance at 9-12 yrs	N=698, n= 146
Fried et al. (2001)	Growth and pubertal milestones at 16 yrs	n=152
Fried and Watkinson (2001)	Facets of attention at 13-16 yrs	N= 698, n=152
Fried et al. (2003)	Cognitive functioning at 13-16 yrs	N= 698, n=145

Maternal Health Practices and Child Development Study (MHPCD)

The Maternal Health Practices and Child Development Study (MHPCD) is a longitudinal cohort that recruited participants from Magee-Womens Hospital in Pittsburgh, PA from 1982 to 1985. Studies from this cohort examined birth, growth, and

neurodevelopmental outcomes associated with prenatal cannabis exposure, and were conducted from 1985 to 2018 in offspring from birth through 22 years of age. These include studies by Day et al., Scher et al., Richardson et al., Cornelius et al., Dahl et al., Chandler et al., Leech et al., Goldschmidt et al., Gray et al., Sonon et al., Willford et al and De Genna et al., as shown in Table D.3 below.

MHPCD comprised three main cohorts: the Teen Mothers cohort (Cornelius et al. 1995), which included mothers younger than 18 years old and their offspring, the prenatal alcohol exposure (PAE) cohort, and the prenatal marijuana exposure (PME) cohort (Day et al. 1991). Additional participants were recruited from 1986 to1987 to participate in select EEG studies (Scher et al. 1988, Dahl et al. 1995, Scher et al. 1998).

Participants for MHPCD were recruited from appointment schedules for the outpatient prenatal clinic. Eligible participants for the PME and PAE cohorts were at least 18 years old and in their fourth month of pregnancy. Women reporting drinking alcohol 3 or more times per week were recruited for the PAE cohort, and the next women who reported drinking less than that amount was also recruited for the control group. Women were invited to participate in the PME cohort if they reported smoking two or more joints per month during the first trimester, and the next women who reported smoking less than that amount was included in the control group (Day et al 1991). Some women were included in both cohorts. Ultimately, 1360 women were interviewed in the first phase of the study (Day et al. 1991). From this combined cohort, random stratified sampling resulted in 829 women. Attrition occurred from 18 deaths, 8 refusals, 16 women missed, 21 moved, 1 adoption, and 2 sets of twins, resulting in 763 live singleton births (Day et al. 1992).

The study's first interview occurred while the participant was in their fourth month of pregnancy and covered substance use in the year prior to pregnancy and during the first trimester (Day et al. 1991). To increase the truthfulness of reports, interviewers employed the bogus pipeline method, in which they convinced the participants that their substance use reports would be confirmed with biological testing, though this was not true (Day et al. 1985). Interviews were performed in a private environment by a trained interviewer to increase the comfort level and thus truthfulness of the participant (Day and Robles 1989). This interview also captured social and demographic information. Additional interviews were performed during the seventh month of pregnancy and during admission for deliver to cover the second and third trimesters (Day et al. 1991). Substance use data was collected for each month of the first trimester of pregnancy, and overall for the second and third trimesters.

To increase the accuracy of participants' recall of substance use the researchers asked about substance use during three periods: from conception to recognition of pregnancy, from recognition to diagnosis of pregnancy, and from the diagnosis to the end of the first trimester (Day and Robles 1989). The researchers used a standardized assessment

protocol, where they used a calendar to ask about specific incidents of use during each period mentioned above. Participants were asked to describe the maximum, minimum, and usual quantities used for each time period, and this information was used to calculate the average number of joints consumed daily (ADJ) (Day et al. 1985). If a participant reported using hash, it was estimated to be equivalent to three joints per bowl, and sinsemilla was counted as 2 joints per use, based on THC potency estimates.

The women selected for PAE and PME cohorts were generally of lower social status. Seventy-four percent of the women had graduated high school, 60% had incomes lower than $400 per month, 67% were single and 32% had only been pregnant once (Day et al. 1992). Approximately half of the sample population were white, and half were black. Overall, 40.3% of women reported marijuana use during their first trimester, 54.3 reported using tobacco, 64.5 used alcohol, and 11 reported use of illicit drugs other than marijuana (Day et al. 1992). Reported use for all substances decreased by the third trimester. Only 17.8% of women reported marijuana use, 52.6% used tobacco, 31.8% consumed alcohol, and 1.3% reported other drug use.

An additional 108 women were recruited for participation in EEG studies (Dahl et al. 1995), presumably using the recruitment and interview methods described above; however, this process was not described in detail. These participants were not part the 1360 women described above and their data is only analyzed in the studies by Scher et al. 1988, Dahl et al. 1995, and Scher et al. 1998.

Women were recruited for the Teen Mother cohort in the same manner as the previous two cohorts (Cornelius et al. 1995). Three hundred and twenty-nine adolescents under 18 years old were recruited in their fourth or fifth months of pregnancy from Magee-Womens Hospital, resulting in 310 live singleton births. Participants for this cohort were interviewed in their fourth or fifth month of pregnancy and again within 24-36 hours of birth (Cornelius et al. 1995).

Table D.3 Maternal Health Practices and Child Development Study (MHPCD) Studies on the effects of Prenatal/Perinatal Exposure to Cannabis

Reference	Outcome, Timing	N, study n
Scher et al. (1988)	Infant sleep and arousal 24-36 hours after delivery	N=1360* n=763 live singleton births n=55 selected for EEG-sleep study
Richardson et al. (1989)	Infant neurodevelopment 24 hours after delivery	n=373 newborns (cohort not mentioned) Only included infants from uncomplicated deliveries without general anesthesia
Day et al. (1991)	BW, BL, PI, SGA, GA, PTB, BD, chest circumference	n=564 from PME cohort only n=519 live singleton births
Day et al. (1992)	Growth from birth to 3 yrs	N=763 8 mos: n=592; 18 mos: n=645; 36 mos: n=672

Reference	Outcome, Timing	N, study n
Day et al. (1994a)	Growth through 6 yrs, palpebral fissures	N=763 n=668
Day et al. (1994b)	Cognitive development at 3 yo	N=763 n=655
Cornelius et al. (1995)	BW, LBW, BL, HC, PI, SGA, BD, chest circumference	N=329 mother/child dyads (Adolescent cohort**) n=310 live singleton births
Dahl et al. (1995)	Sleep disruption at 3 yo	N=763 n=38 19 from PME & PAE cohort; 19 from EEG sample
Richardson et al. (1995)	Cognitive and motor function at 8 and 18 mos	N=763 8 mo: n=592; 18 mo: n=645
Chandler et al. (1996)	Gross motor development at 3 yo	N=763 n=650
Scher et al. (1998)	Visually evoked potentials at birth, 1, 4, 8 and 18 mos	N=108*** Birth: n=22; 1 mo: n=18; 4mo: n=33; 8mo: n=58; 18 mo: n=70
Leech et al. (1999)	Behavior problems at 6 yo	N=763 n=608
Goldschmidt et al. (2000)	Academic achievement at 10 yo	N=763 n=636 interviewed n=575 w/ teachers report
Cornelius et al. (2002)	Growth at 6 yrs	N=445 (Adolescent cohort) n=413 live-born singletons n=345
Richardson et al. (2002)	Cognitive function at 10 yrs	N=763 n=593
Gray et al. (2005)	Depressive symptoms at 10 yrs	N=763 n=636
Day et al. (20060	Cnb use at 14 yrs	N=763 n=563
Leech et al. (2006)	Depression and anxiety at 10 yrs	N=763 n=636
Goldschmidt et al. (2008)	Intelligence at 6 yrs	N=763 n=648
Willford et al. (2010a)	Processing speed, visual-motor coordination, interhemispheric transfer at 16 yrs	N=585 (PAE cohort only) n=45 recruited to MRI study
Willford et al. (2010b)	Caudate volume asymmetry at 18-22 yrs	N=763 n=320 subsample of 18-22 yo n=45
Day et al. (2011)	Delinquent behavior at 14 yrs	N=763 n=525

Reference	Outcome, Timing	N, study n
Goldschmidt et al. (2012)	School achievement at 14 yrs	N=763 524
Day et al. (2015)	Early age of Cnb use	N=763 n=596
De Genna et al. (2015)	Early vaginal intercourse and oral sex by 14 yo	N=413 (Adolescent cohort) n=324
Sonon et al. (2015)	Cnb use at 22 yrs	N=763 n=589 in this analysis
Cornelius et al. (2016)	Alcohol use at 16 yrs	1176 live singleton infants (PAE, PME, Adolescent) n=917
Goldschmidt et al. (2016)	Adult roles at 22 yrs, lifetime conduct disorder at 16 yrs	N=763 n=608
Sonon et al. (2016)	Depressive symptoms at an eary age, early Cnb initiation, Cnb use disorder at 22 yo	N=763 n=590
De Genna et al. (2018a)	Adult electronic cigarette use at 22-33 yo	N=1176 (PAE, PME, Adolescent) n=427
De Genna et al. (2018b)	Adult co-use of tobacco and Cnb at 21-26 yo	N=763 n=603

* 1360 women were interviewed for PAE and PME cohorts. Of these 829 participants were selected via random sampling, resulting in 763 liveborn singleton infants.
** This does not represent the complete adolescent cohort as the study was performed before the full sample was collected.
*** Dahl et al. (1995) states that 108 women were added for participation in the EEG studies.

Birth Outcomes

A variety of birth outcomes have been investigated epidemiologically, including birthwight, low birthweight, birth length, head circumference, small for gestational age, pre-term birth, and mortaility. Table D.4 is a list of the studies of birth outcomes, organized chronologically, for easy reference, showing the endpoints covered by the individual studies. which provides a high level overview of the scope of the dataset. Table D.5 provides more detailed information of each study concerning study design and exposure, organized by the cohorts described above, as well as other researchers. For each study Appendix Table 1.1 provides a much fuller description of study design and results.

Table D.4. Birth Outcomes Assessed in Human Studies of Cannabis Exposure

STUDY	BW (27)*	LBW (17)	BL (15)	HC (18)	PI (4)	SGA/ IUGR (10)	GA (19)	PTB (19)	PRE/PN Mortality (11)	BD (13)	NICU (6)	PN GROWTH (7)	OTHER (24)
Greenland et al. (1982)		X					X						Resuscitation, meconium staining, other obstetrical
Hingson et al. (1982)	X		X	X			X			X			
Gibson et al. (1983)		X				X		X		X			
Greenland et al. (1983)		X											PROM, other obstetrical
Linn et al. (1983)		X						X	SB	X			PROM, placental abruption, fetal distress, other obstetrical
Fried et al. (1984)	X						X						
Tennes et al. (1985)	X		X	X	X		X	X		X		At 1 yr	Infant sex, other obstetrical
Hatch and Bracken (1986)	X	X				X	X	X					
Fried and O'Connell (1987)												At 1 & 2 yrs	
Kline et al. (1987)	X												
Zuckerman et al. (1989)	X		X	X			X			X			

STUDY	BW (27)*	LBW (17)	BL (15)	HC (18)	PI (4)	SGA/ IUGR (10)	GA (19)	PTB (19)	PRE/PN Mortality (11)	BD (13)	NICU (6)	PN GROWTH (7)	OTHER (24)
Frank et al. (1990)					X								Neonatal body proportionality and composition
Day et al. (1991)	X		X		X	X	X	X		X			Chest circumference
Kline et al. (1991)									SAB				
Williams et al. (1991)													Placental abruption
Astley et al. (1992)										X			
Day et al. (1992)												Birth to 3 yrs	
Day et al. (1994)												Through 6 yrs	Palpebral fissures
Kliegman et al. (1994)		X						X					
Knight et al. (1994)	X		X	X			X						
Thompson et al. (1994)						X							
Cornelius et al. (1995[b])	X	X	X	X	X	X	X			X			Chest circumference
Shiono et al. (1995)		X						X					Placental abruption
Berenson et al. (1996)		X		X							X		

STUDY	BW (27)*	LBW (17)	BL (15)	HC (18)	PI (4)	SGA/ IUGR (10)	GA (19)	PTB (19)	PRE/PN Mortality (11)	BD (13)	NICU (6)	PN GROWTH (7)	OTHER (24)
English et al. (1997)	X	X											
Ewing et al. (1997)										VSD			
Fried et al. 1999	X		X	X								Birth to 9-12 yrs	
Fried et al. (2001)												+ Mile-stones 13-16 yrs	.
Klonoff-Cohen and Lam-Kruglick (2001)									SIDS				
Scragg et al. (2001)									SIDS				
Cornelius et al. (2002)												At 6 yrs	
Fergusson et al. (2002)	X		X	X				X	Peri-natal		X		
Quinlivan and Evans (2002)	X		X	X			X	X					BW ratio, PROM, other miscellaneous obstetrical outcomes
Shankaran et al. (2004)	X		X	X									
Williams et al. (2004)										VSD			

STUDY	BW (27)*	LBW (17)	BL (15)	HC (18)	PI (4)	SGA/ IUGR (10)	GA (19)	PTB (19)	PRE/PN Mortality (11)	BD (13)	NICU (6)	PN GROWTH (7)	OTHER (24)
Hurd et al. (2005)													Growth at mid-gestation: weight, length, foot length, occipital frontal HC, PI
Lozano et al. (2007)	X		X	X									
Rivkin et al. (20080				X									Brain volume
Schempf and Strobino (2008)	X	X											
El Marroun et al. (2009)	X			X									Fetal growth mid and late pregnancy: weight, trans-cerebellar diameter
van Gelder et al. (2009)										X			
Gray et al. (2010)	X		X	X			X						
van Gelder et al. (2010)	X	X					X	X					
Dekker et al. (2012)								X					PROM
Janisse et al. (2014)	X						X						Fetal growth: BW "residualized" for GA
Saurel-Cubizolles et al. (2014)	X					X	X	X					
Varner et al. (2014)									SB				

STUDY	BW (27)*	LBW (17)	BL (15)	HC (18)	PI (4)	SGA/ IUGR (10)	GA (19)	PTB (19)	PRE/PN Mortality (11)	BD (13)	NICU (6)	PN GROWTH (7)	OTHER (24)
Chabarria et al. (2016)	X			X				X					PROM, placental abruption
Conner et al. (2016)	X	X				X	X	X	Peri-natal , SAB, SB		X		Placental abruption
Gunn et al. (2016)	X	X	X	X		X	X	X	SAB, peri-natal		X		PROM, placental abruption, jaundice, resuscitation, respiratory distress
Leemaqz et al. (2016)						X		X					
Mark et al. (2016)	X	X					X	X			X		Very LBW
Coleman-Cowger et al. (2018)		X	X	X				X	SAB, SB	X	X		
Massey et al. (2018)	X	X					X						Infant sex
Molnar et al. (2018)													Secretory Immunoglobulin A (Immune function)
Petrangelo et al. (2018)						X		X	SB	X			PROM, other obstetrical
Howard et al. (2019)	X		X	X			X		Peri-natal				Neonatal abstinence syndrome

Table D.5. Birth Outcome: Summary of Selected Study Design and Exposure Elements

Study				Exposure			
Referemce	**Design**	**Location**	**Outcomes/ Sample size**	**Timing**	**Assessment**	**Quantification**	**Covariates**
Ottawa Prenatal Prospective Study (OPPS)							
Fried et al. (1984)	Prospective cohort	Ottawa, Canada	BW, GA n = 583	Each trimester	Self-report by interview	Categorized as: non-users; irregular users (<1 joint/wk or second-hand exposure); moderate users (2-5 joints/wk); heavy users (>5 joints/wk)	Alcohol Other drugs Tobacco
Fried and O'Connell (1987)	Prospective cohort	Ottawa, Canada	Growth at 1 and 2 yrs n = 123	Each trimester	Self-report by interview	*See Fried et al. 1984	Alcohol Other drugs Tobacco
Fried et al. (1999)	Prospective cohort	Ottawa, Canada	BW, BL, HC, growth from birth to 9-12 yrs n = 190	Each trimester	Self-report by interview	*See Fried et al. 1984	Alcohol Other drugs Tobacco
Fried et al. (2001)	Prospective cohort	Ottawa, Canada	Growth and pubertal milestones btwn 13-16 yrs n = 152	Each trimester	Self-report by interview	Categorized as: no use; moderate use (0-6 joints/wk); heavy use (>6 joints/wk)	Alcohol Other drugs Tobacco
Maternal Health Practices and Child Development (MHPCD)							
Day et al. (1991)	Prospective cohort	Pittsburgh, PA	BW, BL, PI, SGA, GA, PTB, BD, chest circumference n = 519	Each trimester	Self-report by interview	Quantity was calculated as average daily joints (ADJ), continuous	Alcohol Other drugs Tobacco
Day et al. (1992)	Prospective cohort	Pittsburgh, PA	Growth from birth to 3 yrs n = 763 at birth n = 672 at 3 yrs	Each trimester	Self-report by interview	*See Day et al. 1991	Alcohol Other drugs Tobacco
Day et al. (1994a)	Prospective cohort	Pittsburgh, PA	Growth through 6 yrs, palpebral fissures n = 668 at 6 yrs	Each trimester	Self-report by interview	*See Day et al. 1991	Alcohol Other drugs Tobacco
Cornelius et al. (1995)	Prospective cohort	Pittsburgh, PA	BW, LBW, BL, HC, PI, SGA, GA, BD, chest circumference n = 310	Each trimester	Self-report by interview	Categorized as: no use; light (0-0.4 joints/day); moderate (0.41-0.88 joints/day); heavy (≥0.89 joints/day)	Alcohol Other drugs Tobacco
Cornelius et al. (2002)	Prospective cohort	Pittsburgh, PA	Growth at 6 yrs n = 345	Each trimester	Self-report by interview	*See Cornelius et al. 1995	Alcohol Other drugs Tobacco
Remaining Studies							
Greenland et al. (1982)	Prospective cohort	Los Angeles, CA	LBW, GA, resuscitation, meconium staining, misc. obstetrical outcomes n = 71	3 mos prior to conception and during preg	Blood sample Urine Self-report by questionnaire	Biological assays performed to confirm self-report assessment	Alcohol Tobacco

Study				Exposure			
Referemce	**Design**	**Location**	**Outcomes/ Sample size**	**Timing**	**Assessment**	**Quantification**	**Covariates**
Hingson et al. (1982)	Retrospective cohort	Boston, MA	BW, BL, HC, GA, BD, FAS-like features n = 1,343 – 1,384	Prior to preg and each trimester	Self-report by interview	Categorized as: non-users; using </mo; 1/mo but <once/wk; 1-2/wk; 3+/wk	Alcohol Psychoactive drug use Tobacco
Greenland et al. (1983)	Prospective cohort	Los Angeles, CA	LBW, PROM, meconium staining, resuscitation, jaundice, misc. obstetrical outcomes n = 313	During preg	Self-report by interview	Participants were classified as Cnb users if they reported any use during preg	Alcohol Tobacco
Gibson et al. (1983)	Prospective cohort	Adelaide, Australia	LBW, IUGR, PTB, BD n = 7,301	During preg	Self-report by interview	Categorized as: no use; ≤once/wk; >once/wk	Alcohol Tobacco
Linn et al. (1983)	Retrospective cohort	Boston, MA	LBW, PTB, SB, BD, PROM, placental abruption, fetal distress, misc. obstetrical outcomes n = 12,424	During preg	Self-report by interview	Categorized as: no use; occasional; weekly; daily	1st trimester alcohol use Tobacco
Tennes et al. (1985)	Prospective cohort	Denver, CO	BW, BL, HC, PI, GA, PTB, BD, growth at 1 yr, infant sex, misc. obstetrical outcomes n = 156	Each trimester	Self-report by interview	Categorized as: no use; light (once ever – 1/wk); moderate (>1/wk but <daily); heavy ≥1/wk)	Amphetamines Alcohol Tobacco
Hatch and Bracken (1986)	Prospective cohort	New Haven, CT	BW, LBW, SGA, GA, PTB n = 3,857	During preg	Self-report by interview	Categorized as: no use; occasional (≤1/mo); regular (2-3/mo)	Alcohol Tobacco
Kline et al. (1987)	Prospective cohort	New York City, NY	BW n = 2,735	Either: in the 3 mos prior to ~20 wks gestation; or 2 mos before the last menstrual period, as well as, during preg	Self-report by questionnaire	Categorized as: no use; <1/mo; 2-4/mo; 2-3/wk; 4-6/wk; daily	Tobacco
Zuckerman et al. (1989)	Prospective cohort	Boston, MA	BW, BL, HC, GA, BD n = 1,226	3 mos before preg and during preg	Urine Self-report	Positive by urine assay Positive by self-report only Negative by self-report and urine	Cocaine Tobacco
Frank et al. (1990)	Prospective cohort	Boston, MA	PI, neonatal body proportionality and composition n = 1,082	During preg	Urine Self-report	Positive urine assay Positive self-report Negative urine and self-report	Alcohol Cocaine Tobacco
Kline et al. (1991)	Case-control	New York City, NY	SAB n = 3,002	During preg	Self-report by interview	Use was assessed as number of days/wk Analyzed as unexposed/exposed	Alcohol Tobacco
Williams et al. (1991)	Nested case-control	Boston, MA	Placental abruption n = 1,400	During preg	Self-report by interview	Categorized as: none; occasional; at least weekly use	Alcohol in 1st trimester Tobacco
Astley et al. (1992)	Retrospective cohort	Seattle, WA	FAS-like features n = 80	During preg	Self-report by interview	Use was assessed as number of days/wk Analyzed as unexposed/exposed	Alcohol Tobacco

Study				Exposure			
Referemce	**Design**	**Location**	**Outcomes/ Sample size**	**Timing**	**Assessment**	**Quantification**	**Covariates**
Kliegman et al. (1994)	Prospective cohort	Cleveland, OH	LBW, PTB n = 425	During preg	Urine Self-report by interview	Exposure was positive based on self-report and/or urine	Alcohol Cocaine Tobacco
Knight et al. (1994)	Prospective cohort	Washington D.C., US	BW, BL, HC, GA n = 349	Each trimester	Serum Some urine	Exposure categorized based on serum concentrations of Cnb metabolite	Other substances excluded due to small sample size
Thompson et al. (1994)	Case-control	New Zealand	SGA n = 1,800	During preg	Self-report by interview	Use during preg was as unexposed/exposed	Alcohol Tobacco
Shiono et al. (1995)	Prospective cohort	OK, NY, LA, TX, WA	LBW, PTB, placental abruption n = 7,470	During preg	Serum assay Self-report by interview	Exposure was positive based on self-report and/or serum	Alcohol Cocaine Tobacco
Berenson et al. (1996)	Retrospective cohort	Galveston, TX	BW, HC, NICU admission n = 238	During preg	Urine Self-report	Exposure assessed based on urine analysis. Analyzed as exposed/unexposed	Alcohol Cocaine Tobacco
English et al. (1997)	Meta-analysis	US, Canada	BW, LBW Did not note 'n'	Each trimester or during preg Varied by analysis	Urine Self-report Varied by study	Use of Cnb was dichotomized for some analysis, or separated by frequency as infrequent (≤1 time/wk) or frequent (≥4 times/wk)	Tobacco
Ewing et al. (1997)	Case-control	Baltimore, MD	Ventricular septal defect n = 491 case infants	Maternal exposure assessed 3 mos before conception through 1st trimester. Paternal exposure assessed 6 mos before conception	Self-report	Exposure assessment was binary. Analyzed as unexposed/exposed	Alcohol Tobacco
Klonoff-Cohen and Lam-Kruglick (2001)	Case-control	California, US	SIDS n = 478	During preg	Self-report by telephone interview	Use was dichotmized for 3 time periods, conception, during preg, postnatally.	Alcohol Tobacco
Scragg et al. (2001)	Case-control	New Zealand	SIDS n = 1,985	During preg	Self-report by telephone interview	Use was assessed as: none; ≥weekly; <weekly Also analyzed as a dichotomous variable	Alcohol Tobacco
Fergusson et al. (2002)	Prospective cohort	Bristol, U.K.	BW, BL, HC, PTB, perinatal mortality, NICU admission n = 12,129	6 mos before conception First 3 mos of preg From 3 mos to date of questionnaire	Self-report by questionnaire	Categorized as: no use; <1/wk before and throughout preg; 1/wk before or during preg but not throughout; at least 1/wk before and throughout preg	Alcohol Hard drugs Tobacco
Quinlivan and Evans (2002)	Prospective cohort	Australia	BW, BL, HC, GA, PTB, BW ratio, PROM, misc. obstetrical outcomes n = 456	During preg	Self-report by interview	Categorized as: no use; Cnb only; multidrug use	Alcohol Illegal drug use Tobacco

Study				Exposure			
Referemce	**Design**	**Location**	**Outcomes/ Sample size**	**Timing**	**Assessment**	**Quantification**	**Covariates**
Shankaran et al. (2004)	Retrospective cohort	FL, RI, TN, MI	BW, BL, HC n = 651	3 mos before preg and each trimester	Meconium Self-report by interview	Use was categorized as: no use; high - (1 to ≥3 joints more often than 1 or 2 days/wk, or <1 joint 1-2 days/wk); moderate - (>1 joint 1-3 days/mo or <1 joint 1-2 days/wk); low - (<1 joint 1-3 days/mo)	Alcohol Cocaine Tobacco
Williams et al. (2004)	Case-control	Atlanta, GA	Ventricular septal defect n = 3,151	3 mos before preg through 1st trimester	Self-report by telephone interview	Categorized as: no use; light (≤2 days/wk); heavy (≥3 days/wk)	Alcohol Other drugs excluded due to small sample size
Hurd et al. (2005)	Retrospective cohort	Brooklyn, NY	Growth at mid-gestation: weight, length, foot length, occipital-frontal HC, PI n = 183	During preg up to elective abortion	Urine Meconium Self-report by interview	Exposure assessed by urine, meconium, or self-report	Alcohol Other drugs Tobacco
Lozano et al. (2007)	Prospective cohort	Barcelona, Spain	BW, BL, HC n = 974	During preg	Meconium	Categorized as unexposed/exposed based on meconium analysis	No covariates
Rivkin et al. (2008)	Prospective cohort	Boston, MA	HC, brain volume n = 123 n = 35 assessed w/ MRI	During preg	Urine Meconium Report from medical records	Exposure assessed based on urine, meconium, or medical records	Alcohol Cocaine Tobacco
Schempf and Strobino (2008)	Retrospective cohort	Baltimore, MD	BW, LBW n = 808	During preg	Urine from medical records. Self-report by interview	Exposure assessed as: none; daily; weekly; monthly Analyzed as unexposed/exposed	Alcohol Other drugs Tobacco
El Marroun et al. 2009	Prospective cohort	Rotterdam, the Netherlands	BW, HC, fetal growth mid-preg: weight; trans-cerebellar diameter n = 7,452	From conception to recognition of preg, and during preg	Self-report by interview	Categorized as: no use daily; weekly; monthly	Alcohol Other drug users excluded
van Gelder et al. (2009)	Case-control	US National Birth Defects Prevention Study	BD n = 15,208	1 mo before preg through 3rd mo preg	Self-report by telephone interview	Categorized as: no use; incidental (≤1 time/wk); moderate (>1 time/wk but <1 time/day); heavy (≥ 1 time/day)	Alcohol Other drugs Tobacco
Gray et al. (2010)	Prospective cohort	Buffalo, NY	BW, BL, HC, GA n = 86	Each trimester and during preg	Meconium Self-report by interview	Categorized as unexposed/exposed based on meconium or self-report	Tobacco
van Gelder et al. (2010)	Retrospective cohort from case-control	US National Birth Defects Prevention Study	BW, LBW, GA, PTB n = 5,661	1 mo before preg through 3rd mo of preg	Self-report by telephone interview	Categorized as: no use; incidental (≤1 time/wk); moderate (>1 time/wk but <1 time/day); heavy (≥ 1 time/day)	Alcohol Other drugs Tobacco

Study				Exposure			
Referemce	**Design**	**Location**	**Outcomes/ Sample size**	**Timing**	**Assessment**	**Quantification**	**Covariates**
Dekker et al. (2012)	Prospective cohort	Auckland, New Zealand; Adelaide, Australia	SPTB – Premature labor w/ rupture of membranes SPTB – Premature labor w/ intact membranes n = 3,190	Before preg or during 1st trimester, at 15 wks gestation	Self-report by interview	Dichotomized for pre-conception, during 1st trimester	Alcohol Other drugs Tobacco
Janisse et al. (2014)	Prospective cohort	Detroit, MI	BW, GA, fetal growth: BW "residualized" for GA n = 3,090	During preg	Self-report by interview	Exposure was expressed as the proportion of prenatal visits during which participant reported Cnb use	Alcohol Tobacco
Saurel-Cubizolles et al. (2014)	Retrospective cross-sectional	Paris, France	BW, SGA, GA, PTB (SPTB, Medically indicated PTB) n = 13,545	During preg	Self-report by interview	Categorized as: no use; <1/mo; ≥1/mo	Alcohol Tobacco
Varner et al. (2014)	Case-control	RI, MA, GA, TX, UT	SB n = 3,506	During preg	Umbilical cord homogenate	Categorized as unexposed/exposed based on umbilical cord homogenate analysis	Alcohol Other drugs Tobacco
Chabarria et al. (2016)	Retrospective cohort	Houston, TX	BW, HC, PTB, PROM, placental abruption n = 12,069	During preg	Self-report by interview	Use was categorized as ever use or current use	Tobacco
Conner et al. (2016)	Meta-analysis of 31 studies	US, Australia, the Netherlands, U.K., Canada, Jamaica, Spain, France, New Zealand	BW, LBW, SGA, GA, PTB, SB, SAB, perinatal mortality, NICU admission, placental abruption n = 132,718	Varied by study	Meconium Umbilical cord Urine Self-report Various combinations per study	Assessment varied by study Data was dichotomized, stratified by tobacco use, or categorized as low/ less than weekly, moderate/weekly, high/daily for separate analyses	Some of the outcomes were stratified by tobacco
Gunn et al. (2016)	Meta-analysis	US, Canada, Australia, the Netherlands, Iran, Jamaica, Spain, Brazil	BW, LBW, BL, HC, SGA, GA, PTB, SAB, perinatal mortality, NICU admission, PROM placental abruption, misc. obstetrical outcomes No 'n' reported	Timing of assessment varied by study	Meconium Hair Serum Urine Self-report Exposure assessment varied by study	Assessment varied by study Data was dichotomized for analysis	Alcohol Other drugs Tobacco
Leemaqz et al. (2016)	Prospective cohort	Australia, New Zealand, Ireland, U.K.	SGA, PTB n = 5,588	Up to 15 wks gestation, up to 20 wks gestation, and during preg	Self-report by interview	Categorized as: no use; quit prior to preg; quit prior to 15 wks gestation; quit prior to 20 wks gestation; still using at 20 wks gestation	Alcohol Tobacco
Mark et al. (2016)	Retrospective cohort	Baltimore, MD	BW, LBW, very LBW, GA, PTB, NICU admission n = 396	During preg	Urine Self-report	Categorized as unexposed/exposed based on self-report and/or urine	Alcohol Tobacco

Study				Exposure			
Referemce	**Design**	**Location**	**Outcomes/ Sample size**	**Timing**	**Assessment**	**Quantification**	**Covariates**
Coleman-Cowger et al. (2018)	Prospective cohort	Baltimore, MD	LBW, BL, HC, PTB, SB, SAB, BD, NICU admission n = 500	1 mo before preg and 1 mo before date of interview During preg	Self-report by interview and questionnaire Hair and urine to validate survey responses	Categorized as: no use of Cnb or tobacco cigarettes; Cnb only; Tobacco cigarettes only; Co-use of Cnb and cigarettes	Alcohol Other drugs Tobacco
Massey et al. (2018)	Meta-analysis	US	BW, LBW, GA, sex of infant n = 1,191	Varied by study	Meconium Self-report by interview Measurement varied by study	Assessment varied by study Data was stratified by tobacco use for analysis	Tobacco
Molnar et al. (2018)	Prospective cohort	Northeastern US	Secretory Immunoglobulin A n = 45	Each trimester	Meconium Saliva Self-report by interview	Average number of joints/day assessed per trimester	Did not include covariates due to small sample size
Petrangelo et al. (2018)	Retrospective cohort	Montreal, Canada	IUGR, PTB, SB, postpartum mortality, BD, PROM, misc. obstetrical outcomes n = 12,578,557	During preg, study did not specify when diagnosis occurred	Diagnosis of Cnb abuse or dependence based on ICD-9 codes	Any previous diagnosis of Cnb abuse or dependence was categorized as exposed	Alcohol Other drug use Tobacco
Howard et al. (2019)	Retrospective cohort	Cincinnati, OH	BW, BL, HC, GA, perinatal mortality, neonatal abstinence syndrome n = 2,173	During preg	Meconium Urine	Categorized as: negative for both tests; positive at initial screening (urine), positive at delivery (meconium); positive for both screenings	Tobacco

Birthweight

Twenty-seven studies examined the association between birthweight (BW) and prenatal cannabis exposure. The table below provides an overview of the results of these studies, as well as basic design elements. For each study, a fuller description of the study design, analytical approaches, exposure characterization and study results is provided in Appendix Table 1.1

Of the 27 studies, twelve reported significant associations between prenatal cannabis use and lower birthweight, adjusted for prenatal tobacco use ((Hingson et al. 1982; Kline et al. 1987), (El Marroun et al. 2009; English et al. 1997; Gray et al. 2010; Howard et al. 2019; Janisse et al. 2014; Zuckerman et al. 1989), (Conner et al. 2016; Gunn et al. 2016; Howard et al. 2019; Massey et al. 2018; Saurel-Cubizolles et al. 2014), and two found significant associations with higher birthweight (Kline, Stein et al. 1987, (Fergusson et al. 2002). Kline et al. (1987) reported significant associations with both lower and higher birthweight. This study used two different questions to assess cannabis exposure in two overlapping phases of their study, and found both increased birthweight associated with occasional (2-4 times/month) cannabis use in the three months before the interview, and decreased birthweight associated with daily cannabis use in a different phase with a different prenatal exposure period (two months before and during pregnancy). They reported lower birthweight for most exposure frequencies >3 times/week, though only one was statistically significant. Fergusson et al.(2002) also reported mixed findings: the lower cannabis exposure groups were statistically significantly associated with higher birthweights than for the unexposed, but the highest cannabis exposure group (cannabis at least once/week) was nonsignificantly associated with lower birthweight.

The remaining thirteen studies found no significant associations for cannabis adjusted for tobacco. Chabarria et al. (2016) showed that co-use of cannabis and tobacco had an adverse association with birthweight greater than the sum of each individual effect, but no significant effect of cannabis alone on birthweight (Fried et al. 1984).

Two studies suggested a dose-response relationship between prenatal cannabis exposure and lower birthweight (Hingson et al. 1982, Saurel-Cubizolles et al. 2014). Hingson et al. (1982) found a significant association with any use, however the largest reduction in birthweight was seen in participants who reported use ≥3 times/week (vs. <3 times/week or never). Saurel-Cubizolles et al. (2014) reported that for both the total sample and the subset that used both cannabis and tobacco, more frequent cannabis exposure (≥once/month compared, <once/month, no use) was associated with lower birthweight. This study was unable to perform any analysis with mothers who used cannabis alone, as there were too few cannabis users who did not also use tobacco.

Of the ten studies published in the past decade, seven reported significantly lower birthweight in infants prenatally exposed to cannabis, adjusted for prenatal tobacco exposure. This may be due in part to the steep increase in the concentration of THC in cannabis in recent decades (ElSohly et al. 2016).

Table D.6. Birthweight: Overview of Findings from Human Studies

Reference	Results	Exposure Assessment and Quantification	Study design Sample Size	Covariates
Hingson et al. 1982	Any: **–105 g (p<0.01)** <3 times/wk: **–95 g (p<0.01)** ≥3 times/wk: **–139 g (p<0.01)**	Self-report No use; <1/month; once/mo but <once/wk; 1-2 times/wk; 3+ times/wk	Retrospective n = 1,343-1,384	Alcohol, psychoactive drugs, tobacco
Fried et al. 1984	Mean BW of heavy users was 52 g less than mean BW of nonusers p=0.598	Self-report No use; irregular; moderate; heavy	Prospective n = 583	Alcohol, tobacco
Tennes et al. 1985	Ns assoc w/ Cnb use (no data reported)	Self-report Weekly amount summed to estimate exposure for entire preg and each trimester	Prospective n = 756	Alcohol, tobacco, amphetamines, cocaine
Hatch and Bracken 1986	Occasional use β=1 g Regular use β=–44 g p=0.40	Self-report Once/mo, to 5 times/day	Prospective n = 3,857	Alcohol, tobacco
Kline et al. 1987	Use 2-4 times/mo in 3 mos prior to interview: **199.1 g**, **p<0.05** Daily use in 2 mos prior to LMP and during preg: **-229.6 g (SE: 79.3) p<0.01**	Self-report at 20 wks gestation None; <1/mo; 2-4/mo; 2-3/wk; 4-6/wk; daily	Prospective n = 2,735	Tobacco
Zuckerman et al. 1989	Positive urine : **-79 g (p=0.04)**	Both urine and self-report Ns with self-report only; urine only	Prospective n = 1,226	Cocaine, tobacco
Day et al. 1991	Cnb use during pregnancy ns assoc w/ BW (no data reported)	Self-report Light; moderate; heavy	Prospective n = 519	Alcohol, tobacco, other drugs
Knight et al. 1994	BW ns assoc w/ any serum cannabinoid concentration	Serum, urine Serum concentration measured during 3rd trimester	Prospective n = 349	No covariates
Cornelius et al. 1995	BW ns assoc w/ Cnb use (no data presented)	Self-report Light; moderate; heavy	Prospective n = 310	Alcohol, cocaine, other drugs, tobacco
English et al. 1997	1st trimester Cnb use: pMD = -**48 (-83,-14)** When assessed by frequency: Use ≥4 times/wk: pMD = -**131 (-209, -52)**	Various	Meta-analysis n = 32,483	Tobacco
Fried et al. 1999	Ns assoc w/ Cnb (no data reported)	Self-report No use; mild/moderate use; heavy use	Prospective n = 679	Alcohol, other drugs, tobacco
Fergusson et al. 2002	Use <1/wk before and throughout preg: **58.60 g (12.91, 165.32)** Use 1/wk before or during preg not but throughout preg: **89.22 g (12.98, 165.30)** Use ≥1/wk before and throughout preg: −84.20 g (−174.70, 6.40)	Self-report Prior to preg; 1st trimester; mid-preg	Prospective n = 12,129	Alcohol, tobacco

Reference	Results	Exposure Assessment and Quantification	Study design Sample Size	Covariates
Quinlivan and Evans 2002	Ns assoc w/ Cnb (no data reported)	Self-report No use, Cnb only; multi-drug	Prospective n = 456	Alcohol, tobacco
Shankaran et al. 2004	Ns assoc w/ Cnb (no data reported)	Self-report, meconium High; moderate; low; no use	Retrospective n = 651	Alcohol, cocaine, tobacco
Lozano et al. 2007	Ns assoc w/ Cnb (no data reported)	Meconium	Prospective n = 974	No covariates
Schempf and Strobino 2008	β = -0.2 (-140.6, 140.2)	Self-report, medical records, urine	Retrospective n = 808	Other drugs
El Marroun et al. 2009	Before preg: ns Early preg: β = **-156.6 (-224.0, -82.2)** Continued use: β = **-96.4 (-152.5, -40.4)**	Self-report Before preg; early preg; continuing in preg; no use	Prospective n = 7,452	Alcohol, tobacco
Gray et al. 2010	Based on meconium samples alone, independent of tobacco: Mean (SD): Cnb -ve: 3429 (544) Cnb +ve**:** 2856 (618) **p<0.001**	Self-report, saliva, meconium	Prospective n = 86	Tobacco
van Gelder et al. 2010	Any Cnb during preg: Overall: -17 < (–90, 56) Non-smokers: -31 (-164,101) Smokers: -14 (-102, 75)	Self-report From 3 mos before preg until birth	Retrospective n = 5,661	Alcohol, tobacco
Janisse et al. 2014	High Cnb exposure alone compared to no use was assoc w/ **55 g lower BW** (statistics not reported)	Self-report High cnb exposure means Cnb use reported in >33% of prenatal visits	Prospective n = 3,090	Alcohol, cocaine, other drugs, tobacco
Saurel-Cubizolles et al. 2014	Total sample: No use: 3303 <1/mo: 3157 ≥1/mo: 3054 Trend test **(p<0.001)** Co-use with tobacco only: no use: 3151 <1/mo: 3016 ≥1/mo: 3010 Trend test **p<0.01**	Self-report No use; <once/mo; ≥once/mo	Retrospective n = 13,545	Alcohol, tobacco
Chabarria et al. 2016	<u>BW <25th percentile</u> Cnb 1.09 (0.61, 1.95) Tobacco 2.09 (1.55, 2.83) Dual use 2.79 (1.55, 5.04)	Self-report During preg	Retrospective n = 12,069	Tobacco
Conner et al. 2016	pMD = **-167g (-245, -90)**	Various	Meta-analysis n = 31 studies	Tobacco
Gunn et al. 2016	pMD = -**109.42g (38.72, 180.12)** I^2=63%	Various	Meta-analysis Not reported	No covariates
Mark et al. 2016	Cnb +ve: 3026 g Cnb –ve: 3089 g p=0.555	Urine	Retrospective n = 170	No covariates
Massey et al. 2018	Any use during preg, adjusted for tobacco use: **−84.37 g (−159.45, −9.28)**	Various	Meta-analysis n = 1,191	Alcohol, other drugs, tobacco

Reference	Results	Exposure Assessment and Quantification	Study design Sample Size	Covariates
Howard et al. 2019	Cnb –ve: 3235 g Cnb +ve initial: 3160 g p=0.089 Cnb +ve delivery: 2785 g **p<0.001** Cnb +ve both: 2925 g **p<0.001**	Urine, meconium Samples collected at 1st prenatal visit and at delivery admission	Retrospective n = 2,173	Tobacco

BW – birthweight; Cnb – Cannabis; LMP – last menstrual period; pMD – pooled mean difference

Low Birthweight

Low birthweight is defined as birthweight less than 2500g. Fifteen studies examined the association between prenatal cannabis exposure and low birthweight (Gibson et al. 1983, Linn et al. 1983, Hatch and Bracken 1986, Kliegman et al. 1994, Cornelius et al. 1995, Shiono et al. 1995, Berenson et al. 1996, English et al. 1997, Schempf and Strobino 2008, van Gelder et al. 2010, Conner et al. 2016, Gunn et al. 2016, Mark et al. 2016, Coleman-Cowger et al. 2018, Massey et al. 2018). (One additional study (Greenland et al.1983) did not present analyzes for the low birthweight data.) The table below provides an overview of the results of these studies, as well as basic design elements. For each study, a more complete description of the study design, analytical approaches, exposure characterization and study results is provided in Appendix Table 1.1.

Of the fifteen studies, two reported statistically significant associations (Hatch and Bracken 1986, Gunn et al. 2016). Hatch and Bracken (1986) was a prospective study that reported an association with low birthweight and any cannabis use during pregnancy, but only among white participants (OR=2.6 (1.1, 6.2)). Gunn et al. (2016) was a meta-analysis that compiled data from seven studies and reported a significant association with LBW (pOR=1.77 (1.04, 3.01)); however, this study did not adjust for tobacco use.

The remaining 13 studies did not observe any significant associations with low birthweight. There was no clear trend in sample size, exposure timing, or detection method to account for the differences in findings between studies.

Table D.7. Low Birthweight: Overview of Findings from Human Studies

Reference	Results	Exposure Assessment	Study design Sample Size
Gibson et al. 1983	Ns associated with Cnb when PTB infants were excluded	Self-report	Prospective n = 7,301
Linn et al. 1983	OR = 1.07 (0.87, 1.31)	Self-report	Retrospective n = 12,424
Hatch and Bracken 1986	Whites **2.6 (1.1, 6.2)** Non-Whites 0.7 (0.6, 1.8)	Self-report	Prospective n = 3,857
Kliegman et al. 1994	OR = 2.28 (0.27, 19.5)	Urine, self-report	Prospective n = 425
Cornelius et al. 1995	Ns assoc with LBW (no data reported)	Self-report	Prospective n = 310
Shiono et al. 1995	OR = 1.1 (0.9, 1.5)	Self-report, serum	Prospective n = 7,470
Berenson et al. 1996	OR = 1.2 (0.5, 2.8)	Self-report	Retrospective No use: 147
English et al. 1997	pOR = 1.09 (0.94, 1.27)	Various: self-report and/or urine	Meta-analysis n = 32,483
Schempf and Strobino 2008	OR = 0.93 (0.55, 1.57)	Self-report, medical records, urine	Retrospective n = 808
van Gelder et al. 2010	Overall: OR = 0.7 (0.3, 1.6) Tobacco smokers: OR = 0.7 (0.3, 2.0)	Self-report	Retrospective n = 5,661
Conner et al. 2016	LBW: OR = 1.16 (0.98, 1.37)	Various: self-report with or without urine, meconium, umbilical cord, serum or oral fluid	Meta-analysis n = 31 studies
Gunn et al. 2016	pOR = **1.77 (1.04, 3.01)** I^2=89%	Various: self-report and/or biologic measures	Meta-analysis n = 24 studies
Mark et al. 2016	LBW: OR = 0.87 (0.3, 2.54) Very LBW: OR = 5.87 (0.9, 38.4)	Self-report or urine	Retrospective n = 170
Coleman-Cowger et al. 2018	OR = 1.0 (0.1, 7.9)	Self-report	Prospective n = 338
Massey et al. 2018	Ns in unadjusted analysis	Various	Meta-analysis n = 1,191

Cnb – cannabis; LBW -- low birthweight; pOR – pooled odds ratio; PTB – preterm birth; ns – not significant

Gestational age

Eighteen studies examined the association between prenatal cannabis exposure and gestational age (GA). The table below provides an overview of the results of these

studies, as well as basic design elements. For each study, a more complete description of the study design, analytical approaches, exposure characterization and study results is provided in Appendix Table 1.1.

Four studies reported that cannabis use was associated with shorter gestation or lower GA (Fried et al. 1984, Cornelius et al. 1995, Saurel-Cubizolles et al. 2014, Howard et al. 2019). Fried et al. (1984) reported that women who used more than five cannabis joints per week gave birth 0.8 weeks earlier than non-users (p=0.0309). Fried et al also reported a dose-dependent relationship of decreasing GA with increasing cannabis exposure among heavy users (Spearman's correlation=-0.4, p=0.05). Cornelius et al. (1995) reported that 1st trimester cannabis use was significantly associated with a 7 day decrease in GA (no p-value reported). In the Saurel-Cubizolles et al. (2014) study, the association with shorter gestation was only significant among non-smokers. Howard et al. (2019) conducted a retrospective study and reported median GA of 39.0 weeks for participants who reported cannabis use at delivery (p=0.008), and participants who reported cannabis use at the both the initial and delivery interviews (p=0.001).

Gray et al. (2010) also reported that a positive meconium test for a cannabis biomarker was associated with lower GA; however, there is a discrepancy with the data reported, which are as follows: the reported median GA was 39 weeks for both exposed and unexposed infants, independent of tobacco results, p=0.012, and the median GA for cannabis-exposed and unexposed infants was 39 weeks when limiting the analysis to those exposed to tobacco, p=0.17.

Tennes et al. (1985) reported a positive association between total number of joints smoked in pregnancy and GA (β=0.09, p=0.03). This analysis was adjusted for race, weight gain, pregnancy complications, and PI (cannabis use was not associated with nicotine use in this study).

The remaining twelve studies did not report significant associations between GA and prenatal cannabis use.

Table D.8. Gestational Age: Overview of Findings from Human Studies

Reference	Results	Exposure Assessment	Study design, Sample Size
Hingson et al. 1982	No association	Self-report	Retrospective n = 1365
Fried et al. 1984	GA assoc w/ Cnb use, **p=0.008** GA of heavy users 0.8 wk shorter than non-users **p=0.0309** Among heavy users, dose-dependent relationship btwn shorter GA and amount of Cnb smoked, Spearman's correlation=-0.4, **p=0.05**	Self-report No use; irregular; moderate; heavy	Prospective n = 583

Reference	Results	Exposure Assessment	Study design, Sample Size
Tennes et al. 1985	GA positively assoc w/ total Cnb use during preg **β=0.09 p=0.03**	Self-report Weekly amount summed to estimate exposure for entire preg and each trimester	Prospective n = 756
Hatch and Bracken 1986	No assoc in unadjusted analysis	Self-report Once/mo, to 5 times/day	Prospective n = 3,857
Zuckerman et al. 1989	No association, no data reported	Urine, self-report Negative; self-report only; urine only	Prospective n = 1,226
Day et al. 1991	No significant findings for GA	Self-report	Prospective n = 519
Knight et al. 1994	Ns assoc at any serum Cnb concentration	Serum, urine Serum concentration measured during 3rd trimester	Prospective n = 349
Cornelius et al. 1995	1st trimester Cnb use **ss assoc w/ decrease of 7 days GA,** no p value reported	Self-report Light; moderate; heavy	Prospective n = 310
Quinlivan and Evans 2002	Ns assoc, no data reported	Self-report No use; Cnb only; multi-drug	Prospective n = 456
Gray et al. 2010	Based on meconium, median GA Cnb +ve: 39 wks Cnb –ve: 39 wks, independent of tobacco smoking status, p=0.01; Among tobacco +ve infants only: Cnb +ve: 39 wks Cnb –ve: 39 wks , p=0.17	Self-report, saliva, meconium	Prospective n = 86
van Gelder et al. 2010	Ns assoc, no data reported	Self-report From 3 mos before preg until birth	Retrospective n = 5,661
Janisse et al. 2014	Ns assoc, no data reported	Self-report Cnb use quantified as proportion of prenatal visits women reported use	Prospective n = 3,090
Saurel-Cubizolles et al. 2014	Trend for lower GA w/ increased consumption in **non-smokers (p<0.01)** smokers (p<0.10)	Self-report No use; <once/mo; ≥Once/mo	Retrospective n = 13,545
Conner et al. 2016	Based on pooled unadjusted estimates: MD: -0.1 wks (-0.5, 0.3)	Various	Meta-analysis n = 31 studies
Gunn et al. 2016	Pooled MD = -0.20 (-0.62, 0.22) I^2=33%	Various	Meta-analysis Not reported
Mark et al. 2016	Unadjusted means: Cnb +ve=38 wks 2 days Cnb –ve=28 wks 6 days p=0.139	Urine	Retrospective n = 170

Reference	Results	Exposure Assessment	Study design, Sample Size
Massey et al. 2018	β=0.14 (-0.13, 0.41) p=0.30	Various	Meta-analysis n = 1,191
Howard et al. 2019	Cnb +ve at delivery: Median = 39.0 wks **p=0.008** Cnb +ve at initial screening & delivery: Median = 39.0 wks **p=0.001**	Urine, meconium Samples collected at 1st prenatal visit and at delivery admission	Retrospective n = 2,173

Cnb – cannabis; GA – gestational age; MD – mean difference; ns – not statistically significant; PTB – preterm birth; ss – statistically significant

Preterm birth

Nineteen studies examined the association between prenatal cannabis exposure and preterm birth (PTB). The table below provides an overview of the results of these studies, as well as basic design elements. For each study, a more complete description of the study design, analytical approaches, exposure characterization and study results is provided in Appendix Table 1.1.

Six studies found statistically significant associations with PTB. Three of these studies were prospective (Gibson et al. 1983, Dekker et al. 2012, Leemaqz et al. 2016), two were retrospective (Saurel-Cubizolles et al. 2014, Petrangelo et al. 2018), and one was a meta-analysis (Conner et al. 2016).

Gibson et al (1983) reported that the increased risk of PTB was attributable to those who used cannabis at least once per week. Dekker et al. (2012) conducted a cohort study specifically to examine risk factors for PTB with or without rupture of membrane, and reported that prenatal cannabis use was significantly associated with increased risk for spontaneous PTB with intact membranes, OR=2.34 (1.22, 4.52), but not with PTB with premature rupture of membranes. Dekker et al. did not adjust for prenatal tobacco smoke exposure because it was not an independent risk factor in their data.

Saurel-Cubizolles et al. (2014) reported that among tobacco users, women who used cannabis one or more times per month were at increased risk of PTB, OR=2.68 (1.16, 6.20). Monthly cannabis use was also associated with PTB in the full sample, OR=2.22 (1.04, 4.74), but this analysis did not account for tobacco use.

Conner et al. (2016) reported significant associations between PTB and prenatal cannabis use in their meta-analysis only for “moderate”/weekly and “heavy”/daily cannabis use, OR=2.04 (1.32, 3.17) and OR=1.73 (1.09, 2.73), respectively.

Leemaqz et al. (2016) categorized cannabis users by the timing of use and found that only participants still using cannabis at 20 weeks’ gestation had significantly increased risk of PTB compared to non-users, OR=5.44 (2.44, 12.11). Like Dekker et al. (2012),

Leemaqz found that although cigarette smoking was associated with PTB, it was not an independent risk factor for PTB in their study and did not require statistical adjustment.

Petrangelo et al. (2018) reported a significant association with PTB, OR=1.40 (1.36, 1.43) in their very large US cohort, despite low prevalence of cannabis exposure based on a restrictive exposure definition (cannabis dependence or abuse in the medical record).

Two studies reported borderline significant associations between prenatal cannabis exposure and PTB (Hatch and Bracken 1986, Shiono et al. 1995). Hatch and Bracken (1986) identified a marginally significant increased risk of PTB only among white women. Shiono et al. (1995) assessed cannabis exposure by self-report and serum screening. When Shiono et al. performed analyses based on serum screening, they observed a slight, marginally significant increase in risk of PTB. The authors note that individuals identified as users by serum samples are more likely to be heavy users, as the window for detection by biological assay is small.

Four of the six studies reporting associations with PTB analyzed more than one level of prenatal cannabis exposure, rather than cannabis use as a binary variable (Gibson et al. 1983, Saurel-Cubizolles et al. 2014, Conner et al. 2016, Leemaqz et al. 2016). Additionally, although Petrangelo et al. (2018) did not quantify cannabis exposure, the exposure definition likely included mainly very heavy cannabis use. Of the 13 studies that did not report significant associations, only one included cannabis exposure in analyses as more than a binary variable (Fergusson et al. 2002).

Table D.9. Preterm Birth: Overview of Findings from Human Studies

Reference	Results	Exposure Assessment	Study design Sample Size
Gibson et al. 1983	Proportion of PTB Nonuser 5.6% ≤once/wk 5.1% >once/wk 25% **p=0.002**	Self-Report Nonuser; ≤once/wk; >once/wk	Prospective n = 7,301
Linn et al. 1983	OR = 1.02 (0.87,1.27)	Self-report Occasional; weekly; daily	Retrospective n = 12,424
Tennes et al. 1985	PTB prevalence 0/31 ≥3 times/wk users of cannabis 7% of nonusers No associations reported	Self-report Weekly amount summed to estimate exposure for entire preg and each trimester	Prospective n = 756
Hatch and Bracken 1986	Among regular use: All races: 1.5 (0.9, 2.5) Whites 1.9 (1.0, 3.9)	Self-report Once/mo to 5 times/day	Prospective n = 3,857
Day et al. 1991	Ns differences in adjusted analysis, no data reported	Self-report Light; moderate; heavy	Prospective n = 519
Kliegman et al. 1994	OR=1.89 (0.34, 10.50)	Urine, self-report Positive by either measure	Prospective n = 425

Reference	Results	Exposure Assessment	Study design Sample Size
Shiono et al. 1995	Overall sample: OR=1.1 (0.8, 1.3) Based on serum only: OR=1.3 (1.0, 1.7) Based on self-report: OR=1.1 (0.9,1.6)	Self-report, serum	Prospective n = 7,470
Fergusson et al. 2002	Ns association any measure of Cnb use, no data reported	Self-report Prior to preg; 1st trimester; mid-preg	Prospective n = 12,129
Quinlivan and Evans 2002	Ns association, no data reported	Self-report No use; Cnb only; multi-drug	Prospective n = 456
van Gelder et al. 2010	Overall: 1.0 (0.6,1.9) Nonsmokers: 0.6 (0.1,1.9) Smokers: 1.2 (0.7, 2.1)	Self-report From 3 mos before preg until birth	Retrospective n = 5,661
Dekker et al. 2012	**SPTB-Intact membrane: OR = 2.34 (1.22, 4.52)** SPTB-PPROM (ns) no data	Self-report Preconception; 1st trimester; 15 wks	Prospective n = 3,234
Saurel-Cubizolles et al. 2014	Total sample: <1/mo 1.62 (0.78, 3.40) >1/mo: 2.22 (1.04, 4.74) Cnb+Tobacco smokers: <1/mo 1.86, (0.64, 5.44) **>1/mo: 2.68 (1.16, 6.20)** No tobacco: <1/mo: 1.24 (0.44, 3.49) >1/mo: no data	Self-report No use; < Once/mo ≥ Once/mo	Retrospective n = 13,545
Chabarria et al. 2016	Cnb: 0.84 (0.35, 3.87) Tobacco: 1.63 (1.12, 2.38) Tobacco & Cnb: 2.56 (1.33, 4.94)	Self-report During preg	Retrospective n = 12,069
Conner et al. 2016	Comparisons to no use: Low use (5 studies): OR=1.09 (0.91, 1.32) Moderate use (5 studies) **OR=2.04 (1.32, 3.17)** High use: (2 studies) **OR=1.73 (1.09,2.73)** Cnb only: 1.25 (0.63, 2.50) Cnb + tobacco: 1.85 (1.21, 2.81) Pooled adjusted RR=1.08 (0.82, 1.43) (4 studies)	Various	Meta-analysis n = 31 studies
Gunn et al. 2016	pOR=1.29 (0.80, 2.08)	Various	Meta-analysis Not reported
Leemaqz et al. 2016	Quit prior to preg: OR=2.23 (0.84, 5.86) Quit prior to 15 wks: OR=1.32 (0.55,3.17) Quit prior to 20 wks: OR=2.76 (0.59, 13.01) Still using at 20 wks: **OR=5.44 (2.44, 12.11)**	Self-report Pre-preg; quit by 15 wks; quit by 20 wks; use at 20 wks	Prospective n = 5588
Mark et al. 2016	UNADJUSTED 17.7% CNB +ve v. 12.0% Cnb –ve; p=0.325	Urine	Retrospective n = 170
Coleman-Cowger et al. 2018	Cnb only: 2.2 (0.8, 5.6) Co-use:1.7 (0.5, 5.8)	Self-report Total days past month use	Prospective n = 338

Reference	Results	Exposure Assessment	Study design Sample Size
Petrangelo et al. 2018	**OR=1.40 (1.36, 1.43)**	Diagnosis of dependence or abuse	Retrospective n = 12,578,557

Cnb – cannabis; pOR – pooled odds ratio; PPROM – preterm premature rupture of membranes

Pre- and Postnatal Mortality

Eleven studies examined associations between prenatal cannabis use and spontaneous abortion (SAB), stillbirth (SB), perinatal mortality, and sudden infant death syndrome (SIDS). These included two prospective cohort studies, three retrospective cohort studies, four case-control studies, and two meta-analyses. Six studies reported only unadjusted results for mortality outcomes, and one study examined paternal cannabis use.

Linn et al. (1983) recruited women who were delivering in 1977-1980 for a retrospective cohort study of various birth outcomes (Linn et al. 1983). Adjusted ORs for SB were not reported because there were too few SBs.

One case-control study examined cannabis use and SAB (Kline et al. 1991). Kline et al karyotyped 960 cases of SAB before 28 weeks to investigate whether there was a relationship between cannabis use during the perifertilization period (2 months before to one month after the last menstrual period) and chromosomally normal and abnormal fetuses (Kline et al. 1991). Cannabis use was analyzed as a continuous and dichotomous variable; however, the proportion of cannabis users in this sample was 8%, and 42% of users reported using cannabis less than once per week. Cannabis use was not associated with SAB. There were no differences among trisomy, monosomy X, triploidy, or other chromosomally aberrant losses and chromosomally normal losses related to cannabis use.

Fergusson et al. (2002) examined various birth outcomes in a prospective cohort study. Women with singleton pregnancies were included after 18 weeks' gestation (Fergusson et al. 2002). Perinatal death, defined as fetal death at 20 weeks or later or death less than seven days after birth, was not associated with cannabis use.

Varner et al. (2014; summarized in a brief narrative instead of a table) conducted a case-control study that examined the risk of SB associated with cannabis use, which was quantified by umbilical cord homogenate levels of THCA (Varner et al. 2014). Varner et al. reported a significantly increased risk of SB among cannabis users: OR = 2.34, (1.13, 4.81), p = 0.021; however, "adjusting for cotinine level reduced the stillbirth [OR] for THCA by greater than 10%". The OR after adjusting for cigarette smoking was not reported.

Conner et al. (2016) conducted a meta-analysis of 31 studies to evaluate maternal cannabis use and adverse neonatal outcomes (Conner et al. 2016). Conner et al reported unadjusted pooled ORs: SB OR=1.74 (1.03, 2.93), SAB OR=1.10 (0.84, 1.44), and perinatal death RR=1.09 (0.62, 1.91); each estimate was based on two studies.

Gunn et al. (2016) conducted a systematic review and meta-analysis using 24 studies to examine effects of prenatal cannabis exposure (Gunn et al. 2016). Gunn et al. did not control for potential confounding variables such as tobacco or alcohol use, noting that as many cannabis users are also tobacco or alcohol users, determining a cannabis-only effect was not possible with the available literature. Gunn et al. reported no significant associations between prenatal cannabis exposure and SAB or perinatal mortality.

Coleman-Cowger et al. (2018) conducted a prospective cohort study of numerous birth outcomes (Coleman-Cowger et al. 2018). Although the frequency of cannabis and other substance use was assessed, the final analysis compared non-users, cannabis-only users, tobacco-only users, and co-users, confirmed with urine and hair sample testing. Prenatal cannabis use compared to no substance use was associated with SB and SAB combined, OR = 12.1 (1.03, 141.8); however, there were only 6 cases. The OR for cannabis and tobacco co-use was 10.1 (0.8, 130.7); an OR for cannabis adjusted for tobacco use was not reported.

Petrangelo et al. (2018) performed a 15-year retrospective cohort study with 12.5 million births, using the ICD-9 codes of US nationwide hospital records (Petrangelo et al. 2018). They found the risk of stillbirth was significantly greater among cannabis users: OR=1.50 (1.39, 1.62), $p<0.0001$. Although the exposure window or quantity could not be determined, the ICD-9 classification of “cannabis abuse” or “cannabis dependence” may have caused pregnancies of only the heaviest cannabis users to be considered exposed.

Howard et al. (2019) conducted a retrospective cohort study with participants delivering in 2013-2014 in a tertiary care setting where the standard of care was for all patients to have a urine drug screen at presentation for prenatal care and at admission for delivery (Howard et al. 2019). Participants were excluded if one of the urine drug screens was not available, or if one indicated drugs other than cannabis. Only unadjusted results were reported for some outcomes, including perinatal mortality. Women who tested positive for cannabis at both the initial screen and at delivery had higher odds of perinatal mortality compared to nonusers, unadjusted OR=5.1 (1.98, 13.16). This study’s strengths include exposure assessment and a recent cohort with (likely) more potent cannabis exposure than in earlier studies.

Two studies examined prenatal cannabis exposure as a risk factor for SIDS. Scragg et al. (2001) conducted a case-control study in New Zealand, recruiting participants in 1987-1990 (Scragg et al. 2001). A case was defined as death between 28 days and one year of age. There were 393 cases and 1592 controls from the same hospitals as

cases. Mothers were interviewed about prenatal cannabis and postnatal cannabis and other substance use. SIDS was non-significantly associated with prenatal cannabis use, OR=1.30 (0.69, 1.87) adjusting for tobacco use, ethnicity, SES, marital status, age at first pregnancy, and infant age. When all potential confounders were included, the association diminished slightly: OR=1.18 (0.76, 1.85). The OR for prenatal cannabis use may be confounded by maternal postnatal cannabis use, which was a slightly stronger predictor of SIDS, and other smoke exposure.

Klonoff-Cohen and Lam-Kruglick (2001) conducted a case-control study to investigate whether maternal and paternal drug use during conception, pregnancy, and postnatally increase the risk of SIDS (defined as death between one week and one year of age) (Klonoff-Cohen and Lam-Kruglick 2001). The sample included 239 SIDS cases and 239 control infants who were born in 1989-1992 in the same hospitals and matched to cases by birth date, sex, and race. Parents were interviewed about use of cigarettes, alcohol, and recreational and other drugs during the conception period (time between last menstrual period and confirmation of pregnancy), pregnancy, and after delivery. Maternal cannabis use during either the conception period or pregnancy, adjusted for prenatal tobacco use, was not associated with SIDS. However, SIDS was associated with paternal cannabis use during the conception period, OR=2.2 (1.2, 4.2), and pregnancy, OR=2.0 (1.0, 4.1), adjusted for paternal postnatal tobacco smoking and alcohol use during conception. There were no interactions among paternal drug use, smoking, and drinking, and maternal and paternal recreational drug use.

Head Circumference

Seventeen studies (Tennes et al. 1985, Zuckerman et al. 1989, Day et al. 1991, Knight et al. 1994, Cornelius et al. 1995, Berenson et al. 1996, Fergusson et al. 2002, Quinlivan and Evans 2002, Shankaran et al. 2004, Lozano et al. 2007, Rivkin et al. 2008, El Marroun et al. 2009, Gray et al. 2010, Chabarria et al. 2016, Gunn et al. 2016, Coleman-Cowger et al. 2018, Howard et al. 2019) examined the association between prenatal cannabis exposure and head circumference. The table below provides an overview of the results of these studies, as well as basic design elements. For each study, a fuller description of the study design, analytical approaches, exposure characterization and study results is provided in Appendix Table 1.1

Of the 17 studies, two prospective studies found statistically significant associations (El Marroun et al. 2009, Gray et al. 2010). Coleman-Cowger et al. (2018) also found a significant association, but only when cannabis was co-used with tobacco (OR = 5.7 (1.1, 28.9)).

El Marroun et al. (2009) examined fetuses in early and late pregnancy using ultrasound. The study showed that exposure to cannabis prior to pregnancy or in early pregnancy

was associated with reduced head circumference growth of 0.1 (0.2, 0.02) mm/wk, for either time period of exposure.

Gray et al. (2010) assessed prenatal cannabis exposure with meconium samples. The study showed that prenatal cannabis use reduced mean head circumference by 1.4 cm (p=0.003), independent of tobacco smoking status. This association remained significant when analyzed for women who co-used tobacco and cannabis during pregnancy.

The remaining 14 studies did not show a significant association with prenatal cannabis exposure and head circumference. Of these 14 studies, 10 were prospective (Tennes et al. 1985, Zuckerman et al. 1989, Day et al. 1991, Knight et al. 1994, Cornelius et al. 1995, Fergusson et al. 2002, Quinlivan and Evans 2002, Lozano et al. 2007, Rivkin et al. 2008, Coleman-Cowger et al. 2018), four were retrospective (Berenson et al. 1996, Shankaran et al. 2004, Chabarria et al. 2016, Howard et al. 2019), and one was a meta-analysis (Gunn et al. 2016).

All but three (Knight et al. 1994, Lozano et al. 2007, Gunn et al. 2016) of the studies adjusted for tobacco use. There was no consistent trend in sample size among studies that found significance; El Marroun et al. (2009) had 7,452 participants, and Gray et al. (2010) had 86 participants. Though Gunn et al. (2010) used meconium to assess exposure, which may be a more reliable measure than other exposure metrics, three other studies (Shankaran et al. 2004, Lozano et al. 2007, Howard et al. 2019) also used meconium and did not show a significant association.

Table D.10. Head Circumference: Overview of Findings from Human Studies

ReferenceStudy Design	Results	Exposure Assessment and Quantification	Sample SizeCnb only	Covariates
Tennes et al. 1985 **Prospective**	Cnb use ns assoc w/ HC (no data reported)	Self-report Weekly amount summed to estimate exposure for entire preg and each trimester	n = 756 Cnb users: 258 31 reported use ≥3 times/week	Alcohol Tobacco Amphetamines Cocaine
Zuckerman et al. 1989 **Prospective**	Cnb use ns assoc w/ HC (no data reported)	Urine, self-report. Negative; self-report only; urine only	n = 1,226 Urine: 202 Self-report only: 129 Negative: 895	Cocaine Tobacco
Day et al. 1991 **Prospective**	Cnb use ns assoc w/ HC (no data reported)	Self-report Light; moderate; heavy	n = 519	Alcohol Tobacco Other drugs

ReferenceStudy Design	Results	Exposure Assessment and Quantification	Sample SizeCnb only	Covariates
Knight et al. 1994 **Prospective**	Cnb ns assoc w/ any serum concentration of cannabinoids	Serum, urine Serum concentration measured during 3rd trimester	n = 349	No covariates
Cornelius et al. 1995 **Prospective**	Cnb use ns assoc w/ HC (no data reported)	Self-report Light; moderate; heavy	n = 310	Alcohol Cocaine Other drugs Tobacco
Berenson et al. 1996 **Retrospective**	Small HC: OR = 0.8 (0.3, 1.8)	Self-report, urine	n = 36 Cnb only	Alcohol Tobacco
Fergusson et al. 2002 **Prospective**	Cnb use ns assoc w/ HC (no data reported)	Self-report Prior to preg; 1st trimester; mid-preg	n = 12,129 6 mos before preg: 585 1st trimester: 311 Mid-preg: 250	Alcohol Tobacco
Quinlivan and Evans 2002 **Prospective**	Cnb use ss assoc w/ 0.4 reduction in HC (test for trend p=0.08)	Self-report No use, Cnb only; multi-drug	n = 456 Cnb only: 62	Alcohol Tobacco
Shankaran et al. 2004 **Retrospective**	High Cnb use ns assoc w/ decrease in HC Moderate, low, and decreasing use ns assoc with increase in HC	Self-report, meconium High; moderate; low; no use	n = 651	Alcohol Cocaine Tobacco
Lozano et al. 2007 **Prospective**	Cnb use ns assoc w/ HC (no data reported)	Meconium	n = 974 Cnb: 52	No covariates
Rivkin et al. 2008 **Prospective**	Cnb use ns assoc w/ a decrease in HC (no data reported)	Self-report	n = 35	Alcohol Cocaine Tobacco
El Marroun et al. 2009 **Prospective**	HC of fetuses exposed to Cnb before preg and in early preg grew **0.1 (0.2, 0.02)** and **0.1 (.2, 0.02)** mm/wk less, respectively, than fetuses of nonusers	Self-report Before preg; early preg; continuing in preg; no use	n = 7,452	Alcohol Tobacco
Gray et al. 2010 **Prospective**	Mean HC, independent of tobacco status, based on meconium: Cnb -ve: 34.4 cm	Self-report, saliva, meconium	n = 86	Tobacco

ReferenceStudy Design	Results	Exposure Assessment and Quantification	Sample SizeCnb only	Covariates
	Cnb +ve: **33.0 cm (p=0.003)** **Results remained ss among tobacco +ve mothers**			
Chabarria et al. 2016 **Retrospective**	HC <25th percentile: Cnb only: OR = 1.44 (0.82, 2.53) Tobacco only: OR = 1.67 (1.20, 2.33) Dual use: OR = 2.34 (1.27, 4.31)	Self-report During preg	n = 12,069 Cnb only: 58 Tobacco & Cnb: 48	Tobacco
Gunn et al. 2016 **Meta-analysis**	pMD = -0.31 (-0.74, 0.13) I^2=97%	Various	Not reported	No covariates
Coleman-Cowger et al. 2018 **Prospective**	Low HC: Cnb-only: OR = 2 .0 (0.4, 10.6) Co-use w/ tobacco: **OR = 5.7 (1.1, 28.9)**	Self-report <1 day/wk; 1-2 days/wk; 3-6 days/wk; daily	n = 338	Alcohol Other drugs Tobacco
Howard et al. 2019 **Retrospective**	Cnb use ns assoc w/ HC Cnb –ve: 13.4 cm Cnb +ve: 13.2 cm	Urine, meconium Samples collected at 1st prenatal visit and at delivery admission	n = 2,173	Tobacco

Birth Length

Fourteen studies examined the association between prenatal cannabis exposure and birth length. The table below provides an overview of the results of these studies, as well as basic design elements. For each study, a fuller description of the study design, analytical approaches, exposure characterization and study results is provided in Appendix Table 1.1.

Of the 14 studies, five studies found statistically significant negative associations (Tennes et al. 1985, Zuckerman et al. 1989, Day et al. 1991, Gray et al. 2010, Howard et al. 2019), while one study found a significant positive association (Fergusson et al. 2002). The remaining eight studies did not find any significant associations with birth length (Knight et al. 1994, Cornelius et al. 1995, Fried et al. 1999, Quinlivan and Evans 2002, Shankaran et al. 2004, Lozano et al. 2007, Gunn et al. 2016, Coleman-Cowger et al. 2018).

Four of five studies with significant negative associations were prospective (Tennes et al. 1985, Zuckerman et al. 1989, Day et al. 1991, Gray et al. 2010). Tennes et al. (1985) assessed exposure for each trimester via self-report and reported that first trimester cannabis use, but not second or third trimester use, was associated with -0.07cm (p=0.001) average birth length. Zuckerman et al. (1989) reported a decrement in birth length of 0.52 cm (p=0.02) associated with prenatal cannabis exposure, identified via maternal urine sample. Day et al. (1991) reported that cannabis use during the first two months of pregnancy was associated with reduced birth length but did not present any adjusted data. Gray et al. (2010) assessed prenatal cannabis exposure with meconium samples and reported that, independent of tobacco exposure, cannabis use was associated with a reduction in mean birth length by **2 cm (p=0.01)**. This association remained significant when analyzed for participants who co-used tobacco and cannabis during pregnancy.

Howard et al. (2019) was a retrospective study that identified prenatal cannabis use with urine and meconium. The study reported that participants with positive urine or meconium samples at the initial screening and at delivery had infants with significantly reduced birth length, but did not report any data.

Fergusson et al. (2002) examined the association with birth length via self-report prior to pregnancy, during the first trimester, and mid-pregnancy. The study reported that cannabis use more than once per week during but not throughout pregnancy was associated with significantly increased birth length, but did not report any data.

The remaining eight studies did not find a significant association between prenatal cannabis exposure and birth length. Six of these studies were prospective (Knight et al. 1994, Cornelius et al. 1995, Fried et al. 1999, Quinlivan and Evans 2002, Lozano et al. 2007, Coleman-Cowger et al. 2018), one was retrospective (Shakaran et al. 2004), and one was a meta-analysis (Gunn et al. 2016).

All but three of the 14 studies (Knight et al. 1994, Lozano et al. 2007, Gunn et al. 2016) adjusted for prenatal tobacco use. There were a wide range of sample sizes, and no clear trend among studies that found significance. There was no study design or exposure detection method that was more likely to report significant results.

Table D.11. Birth Length: Overview of Findings from Human Studies

Reference Study Design	Results	Exposure Assessment and Quantification	Sample Size Cnb only	Covariates
Tennes et al. 1985 **Prospective**	1st trimester use ss assoc with **-0.07 cm (p=0.001)** ns in 2nd and 3rd trimester	Self-report Weekly amount summed to estimate exposure for entire preg and each trimester	n = 756 Cnb users: 258 31 reported use ≥3 times/week	Alcohol Tobacco Amphet-amines Cocaine
Zuckerman et al. 1989 **Prospective**	Positive urine assay: -**0.52 cm (p=0.02)**	Urine, self-report. Negative; self-report only; urine only	n = 1,226 Urine: 202 Self-report only: 129 Negative: 895	Cocaine Tobacco
Day et al. 1991 **Prospective**	Use in first 2 mos preg ss assoc w/ BL (no data presented) Means: Non-user = 49.4 cm 1st trimester = **48.9 cm (p≤0.04)**	Self-report Light; moderate; heavy	n = 519	Alcohol Tobacco Other drugs
Knight et al. 1994 **Prospective**	Cnb ns assoc w/ any serum concentration of cannabinoids	Serum, urine Serum concentration measured during 3rd trimester	n = 349	No covariates
Cornelius et al. 1995 **Prospective**	Cnb ns assoc w/ BL (no data reported)	Self-report Light; moderate; heavy	n = 310	Alcohol Cocaine Other drugs Tobacco
Fried et al. 1999 **Prospective**	No adjusted data reported, ns differences between Cnb use categories	Self-report No use; mild/moderate use; heavy use	n = 679 at birth	Alcohol Other drugs Tobacco
Fergusson et al. 2002 **Prospective**	Use <1/wk before or during, but not throughout preg **ss assoc w/ increased BL** (no data reported)	Self-report Prior to preg; 1st trimester; mid-preg	n = 12,129 6 mos before preg: 585 1st trimester: 311 Mid-preg: 250	Alcohol Tobacco
Quinlivan and Evans 2002 **Prospective**	Cnb use ns assoc w/ BL (no data reported)	Self-report No use, Cnb only; multi-drug	n = 456 nb only: 62	Alcohol Tobacco
Shankaran et al. 2004 **Retrospective**	High and moderate Cnb use ns assoc w/ BL Low and decreaseing Cnb use assoc w/ ns increase in BL	Self-report, meconium High; moderate; low; no use	n = 651	Alcohol Cocaine Tobacco
Lozano et al. 2007 **Prospective**	Cnb use ns assoc w/ BL (no data reported)	Meconium	n = 974 Cnb: 52	No covariates
Gray et al. 2010 **Prospective**	Mean BL independent of tobacco status, based on meconium: Cnb -ve: 50.8cm Cnb +ve: **48.8 cm (p=0.01)**	Self-report, saliva, meconium	n = 86	Tobacco

Reference Study Design	Results	Exposure Assessment and Quantification	Sample Size Cnb only	Covariates
	Results remained ss among tobacco +ve mothers			
Gunn et al. 2016 **Meta-analysis**	pMD = -0.10 (-0.65, 0.45) I^2=59%	Various	Not reported	No covariates
Coleman-Cowger et al. 2018 **Prospective**	Low BL: Cnb-only: OR = 2.0 (0.3, 7.9) Co-use w/ tobacco: OR = 1.4 (0.1, 14.2)	Self-report <1 day/wk; 1-2 days/wk; 3-6 days/wk; daily	n = 338	Alcohol Other drugs Tobacco
Howard et al. 2019 **Retrospective**	Cnb use reported both at initial interview and at delivery ss assoc with **reduced BL p<0.001**	Urine, meconium Samples collected at 1st prenatal visit and at delivery admission	n = 2,173	Tobacco

Birth defects

Thirteen epidemiologic studies – six prospective cohort (Gibson et al. 1983, Tennes et al. 1985, Zuckerman et al. 1989, Day et al. 1991, Cornelius et al. 1995, Coleman-Cowger et al. 2018), four retrospective cohort (Hingson et al. 1982, Linn et al. 1983, Astley et al. 1992, Petrangelo et al. 2018), and three case-control (Ewing et al. 1997, Willimas et al. 2004, van Gelder et al. 2009) studies evaluated associations between prenatal cannabis exposure and birth defects. For each study, a full description of the study design, analytical approaches, exposure characterization and study results is provided in Appendix Table 1.1.

The exposures evaluated were maternal and paternal prenatal cannabis use, cannabis smoke specifically, and cannabis and tobacco co-use, and authors described their outcomes of interest as major malformations (including congenital heart disease, spina bifida, and hypospadias), minor malformations (including undescended testis, hemangiomata and lymphangiomata), ventricular septal defects (VSD), fetal alcohol syndrome (FAS)-like features, or unspecified birth defects.

Eight studies found no significant associations between cannabis use and birth defects (Linn et al. 1983, Gibson et al. 1983, Tennes et al. 1985, Zuckerman et al. 1989, Day et al. 1991, Astlye et al. 1992, Cornelius et al. 1995, Petrangelo et al. 2018). Linn et al. (1983) reported that major malformations were more prevalent among cannabis users, but the OR of 1.36 (0.97, 1.91) was not significant. In Gibson et al. (1983), only 36 (0.5%) of participants used cannabis more than once per week. Cornelius et al. (1995) found that first trimester cannabis use by white women under 16 years of age was associated with marginally significantly increased risk of minor physical anomalies: OR 3.2 (1.0, 10.2). Petrangelo et al. (2018) reported no association with birth defects: OR=1.00 (0.88, 1.13). Although Petrangelo et al. (2018) had an extremely large

sample, cannabis exposure assessment was based on diagnosis of cannabis abuse or dependence in the medical record, the prevalence of which was 0.5%.

Williams et al. (2004) conducted a case-control study focused specifically on maternal lifestyle risk factors for isolated, simple VSD and found a positive association with self-reported frequency of cannabis use in the periconceptional period (three months prior to pregnancy through the first trimester): ordinal OR=1.90 (1.29, 2.81) for light use (≤2 days/wk) compared to no use, and heavy use (≥3 days/wk) compared to light use, suggesting greater risk at higher exposures. Maternal heavy alcohol use and cigarette use were not associated with VSD, but children of mothers who reported heavy alcohol use and cannabis use were at increased risk of VSD: OR=7.51 (2.40, 23.55). The sample was relatively small, with 20 of 122 cases and 118 of 3,009 controls exposed to cannabis. Ewing et al. (1997) reported an increased risk of isolated membranous VSD with paternal cannabis use in the six months before conception: OR=1.36 (1.05, 1.76), adjusted for paternal cocaine/age interaction, maternal age, maternal cocaine use, infant sex, and race. Maternal cannabis use and use of cigarettes and alcohol by either parent were not significantly associated with VSD.

Van Gelder et al. (2009) also used a case-control design to examine the effects of maternal illicit drug use on 20 types of birth defects, and reported a positive association between anencephaly and cannabis use in the first month of pregnancy: OR=2.5 (1.3, 4.9). Gastroschisis was also associated with cannabis use in unadjusted models, but the association was not statistically significant in adjusted models: OR=1.3 (0.9, 1.8). This study had a large sample (n=15,208, including 420 exposed cases) and examined self-reported cannabis exposures in etiologically relevant periods, but made multiple comparisons. Coleman-Cowger et al. (2018) found a significant association between birth defects and co-use of cannabis and tobacco compared to no-use: OR=3.1 (1.2, 8.3), but no significant association with cannabis-only exposure: OR=1.2 (0.5, 2.9), or tobacco-only exposure: OR=1.9 (0.7, 5.3).

Two studies examined FAS-like features. Hingson et al. (1982) reported that women who smoked cannabis during pregnancy “were five times more likely than nonusers (2.0, 12.7) to deliver a child with [FAS-like] features ($p<.001$)”, whereas drinking ≥2 drinks daily was associated with non-significantly decreased risk compared with nondrinkers. Astley et al. (1992) reported no associations between cannabis use and FAS-like features in their small (n=80) study.

Of the nine studies whose exposure periods included the 1960s, 1970s, and 1980s, when Cnb was much less potent than in more recent decades (El Sohly 1984, ElSohly et al. 2016), only Ewing et al. (1997) reported statistically significant associations with birth defects (VSD). The studies reporting statistically significant associations between Cnb and birth defects focused on birth defects rather than multiple types of birth

outcomes, and assessed exposure during early pregnancy ((Williams et al. 2004), (van Gelder et al. 2009)) and spermatogenesis (Ewing et al. 1997).

NICU Admission

Six studies examined the association between prenatal cannabis use and NICU admission. For each study, a description of the study design, analytical approaches, exposure characterization and study results is provided in Appendix Table 1.1.

Although NICU admission was a secondary outcome in many studies that primarily focused on other birth outcomes, such as preterm birth or birth weight, only one reported a significant association (Gunn et al. 2016). Gunn et al. (2016) performed a meta-analysis that reported a pooled OR of 2.02 (95% CI 1.27, 3.21) for infants exposed to cannabis in utero. This finding was calculated by random-effects modelling since heterogeneity was not significant ($p \leq 0.10$, but $I^2 \leq 50\%$). Three of the four studies included in Gunn et al. (2016) were not included in the present document due to a small sample of cannabis users (Gargari et al. 2012) or binary exposure assessment (Bonello et al. 2014 and Hayatbakhsh et al. 2012). The fourth study found a non-significant association between prenatal cannabis exposure and NICU admission (Berenson et al., 1996). In the meta-analyses Gunn et al. did not adjust for tobacco use or other potential confounders. Another meta-analysis (Connor et al. 2016), included 5 studies examining NICU admission, and reported an increased pooled unadjusted estimate (RR = 1.41 (95% CI 0.99, 20). However, adjusted estimates were not available.

The four other studies that did not find significant results included two retrospective cohorts (Mark et al., 2016; Berenson et al.), two prospective cohorts (Fergusson et al., 2002; Coleman-Cowger et al., 2018). All cohort studies assessed cannabis use by self-report, but some studies additionally collected urine or hair samples for toxicological screening (Mark et al., 2016 and Coleman-Cowger et al., 2018). Fergusson et al. (2002), and Coleman-Cowger et al. (2018) gathered information on the frequency of maternal cannabis use, but only Fergusson analyzed these categories in an adjusted model.

Overall, the five non-significant studies used a higher standard of exposure assessment than the study that reported a significant association between prenatal cannabis exposure and NICU admission.

NICU/Special Care Admission

Six studies examined the association between prenatal cannabis use and NICU admission. Although NICU admission was a secondary outcome in many studies that primarily focused on other birth outcomes, such as preterm birth or birth weight, only one reported a significant association (Gunn et al. 2016). Gunn et al. (2016) performed a meta-analysis that reported a pooled OR of 2.02 (95% CI 1.27, 3.21) for infants

exposed to cannabis in utero. This finding was calculated by random-effects modelling since heterogeneity was not significant ($p \leq 0.10$, but $I^2 \leq 50\%$). Three of the four studies included in Gunn et al. (2016) were not included in the present document due to a small sample of cannabis users (Gargari et al. 2012) or binary exposure assessment (Bonello et al. 2014 and Hayatbakhsh et al. 2012). The fourth study found a non-significant association between prenatal cannabis exposure and NICU admission (Berenson et al., 1996). In the meta-analyses Gunn et al. did not adjust for tobacco use or other potential confounders. Another meta-analysis (Connor et al. 2016) included five studies examining NICU admission, and reported a non-significantly increased pooled unadjusted estimate (RR = 1.41 (95% CI 0.99, 20). However, adjusted estimates were not available.

The four other studies that did not find significant results were performed in two retrospective cohorts (Mark et al., 2016; Berenson et al. 1996) and two prospective cohorts (Fergusson et al., 2002; Coleman-Cowger et al., 2018). All cohort studies assessed cannabis use by self-report, and two studies additionally collected urine or hair samples for toxicological screening (Mark et al., 2016 and Coleman-Cowger et al., 2018). Fergusson et al. (2002) and Coleman-Cowger et al. (2018) gathered information on the frequency of maternal cannabis use, but only Fergusson analyzed these categories in an adjusted model. Only one study did not consider prenatal tobacco use as a potential confounder (Mark et al. 2016).

Postnatal Growth

Seven epidemiologic studies evaluated the effects of prenatal cannabis exposure on postnatal growth. All seven were prospective cohort studies and examined the association of cannabis exposure throughout pregnancy and postnatal growth. Growth measures included height, weight, head circumference, body mass index (BMI), ponderal index (PI), skinfold thickness, and weight-for-height *Z* score. Age at follow-up ranged from one to sixteen years. All studies based exposure on self-report.

In a prospective study of birth outcomes, Tennes et al. (1985) conducted a sub-analysis of 129 1-yr-old children of randomly selected women who had been heavy, moderate, or non-users of cannabis during pregnancy. Weight and height at one year were not different among children of heavy, moderate, and non- cannabis users. The sample represented a lower to lower-middle SES population.

Fried and O'Connell (1987) examined associations between heavy cannabis use (≥5 joints/week) and height and weight at 12 and 24 months of age in the OPPS cohort. Only outcomes at 24 months were statistically significant and are reported here. Cannabis use in the year before pregnancy was associated with an increase in weight of 530.17 g ($p < 0.01$). Heavy cannabis use during the first trimester was associated with an increase of 539.16 g ($p = 0.02$), and throughout pregnancy with an increase of

596.69 g (p = 0.01). Third trimester heavy cannabis use was associated with an increase of 623.16 g (p = 0.02). Heavy cannabis use throughout pregnancy was associated with a 1.1 cm increase in height (p = 0.05.).

In another OPPS study, Fried et al. (1999) reported an association between heavy prenatal cannabis use (at least 6 joints per week) and reduced head circumference at 9-12 years ($p \leq 0.05$), but no association with weight at 12 or 48 months. The authors mention the potential influence of maternal caloric intake on child caloric intake, as the marijuana users in their study consumed more calories and protein overall, which could impact lifestyle choices of their children to do the same. This could lead to an increase in weight in the children, though no significant association was found with prenatal exposure in their study.

In the third OPPS study, Fried et al. (2001) examined growth measurements in 152 adolescents at 13-16 years. There were no differences in weight, height, or PI. Heavy use was marginally associated with head circumference (p=0.08), but this relationship was weaker when prenatal alcohol and nicotine exposure were considered (p=0.11).

Three studies from the MHPCD cohort evaluated postnatal growth. All of these studies took into consideration current maternal exposure to cannabis, tobacco, or alcohol use, in addition to prenatal exposures to tobacco and alcohol. Day et al. (1992) and Day et al. (1994a) assessed weight, height, and head circumference at three and six years, respectively, and reported no associations with prenatal cannabis exposure.

The third MHPCD study, by Cornelius et al. (2002), reported a decrease in height of 1.1 inches at six years with any prenatal cannabis exposure in the second trimester ($p < 0.01$). Prenatal cannabis use was not associated with weight, skinfold thickness, head circumference, BMI, PI, or weight-for-height *Z* score at 6 years. The authors note that the association between height and prenatal cannabis exposure is not consistent with other studies, but do not address possible reasons for the inconsistency with the MHPCD or other studies. The number of mothers who used cannabis in the second trimester was 13 (total n=345).

In the OPPS cohort, heavy prenatal cannabis use was associated with heavier and taller children at 24 months ((Fried and O'Connell 1987), reduced head circumference at 9-12 years ((Fried et al. 1999), and a non-significant reduction in head circumference (Fried et al. 2001) at 13-16 years; differences in body size did not persist. The OPPS cohort is predominately middle SES, low-risk cohort. Results from the MHPCD cohort, a high-risk cohort of adolescent mothers, are not consistent with the OPPS findings, or within studies from the same cohort. Two of the three MHPCD studies reported no differences in weight, height, and head circumference at three and six years. The third study also reported no differences in weight, head circumference, and measures of adiposity and proportionality, but diverged from the previous studies in reporting

significantly shorter stature for six-year-old children with prenatal 2nd trimester cannabis exposure.

Neurodevelopment

Studies Available and Tests Used to Assess Neurodevelopment

Studies

A number of epidemiololgical studies have been investigated the effect of cannabis exposure during pregnancy on neurodevelopmental outcomes in offspring. Table D.12 some of the endpoints covered by studies in the first year of life and Table D.13, showing the range of endpoints covered by the individual studies. Table D.14 provides more detailed information of each study concerning study design and exposure, organized by the cohorts described above, as well as other researchers. For each study Appendix Table 1.1 provides a much fuller description of study design and results.

Table D.12 Overview of Epidemiological Studies of Neurodevelopmental Outcomes in Infancy

INFANCY	BNBAS	NNNS	CNS MATURATION	OTHER
Fried 1980 *(OPPS cohort)*	X		Visual response, habituation, tremors, startles	
Fried 1982 *(OPPS cohort)*	X		Visual stimuli responsiveness, tremors, startles, habituation	
Tennes et al. 1985	X			Infant muscle tone
Fried and Makin 1987 (OPPS Cohort)	X		Startles, response to light, habituation, tremors, irritability	
Fried et al. 1987 (OPPS cohort)				Infant state, resting posture, movements, reflexes at 9 and 30 days (Prechtl)
Hayes et al. 1988	X			
Scher et al. 1988 (MHPCD cohort)			Disturbance in neonatal sleep cycling	
Richardson et al. 1989 (MHPCD cohort)	X			
Parker et al. 1990				Jitteriness in infants
Scher et al. 1998 (MHPCD Cohort)			Visually evoked potentials at 1,4,8, and 18 mos	
De Moraes Barros et al. 2006		13 subsets of NNNS		

INFANCY	BNBAS	NNNS	CNS MATURATION	OTHER
Stroud et al. 2018		Self-regulation, handling, lethargy, attention of NNNS		

Italicized studies are summarized in paragraphs

BNBAS- Brazelton Neonatal Behavioral Assessment Scale; NNNS – NICU Network Neurobehavioral Scale, MHPCD -Maternal Health

Practices and Child Development Project cohort; OPPS - Ottawa cohort referred to as the Ottawa Prenatal Prospective Study

Table D.13. Human Studies of Neurodevelopmental Outcomes in Childhood or Adulthood After in Utero or Perinatal Cannabis or Δ^9-THC

	ATTENTION	INTELLIGENCE	NEURO-IMAGING	CNS MATURATION	VISUAL-SPATIAL PROCESSING	BEHAVIOR	PSYCHIATRIC SYMPTOMS	SUBSTANCE USE	OTHER
EARLY CHILDHOOD (1-5 YRS)									
Fried and Watkinson 1988 (OPPS)		Mental & language development at 1 and 2 yrs							Motor development at 2 yrs
Fried and Watkinson 1990 (OPPS)		Cognitive functioning at 3 and 4 yrs							
Day et al. 1994 (MHPCD)		Cognitive develop-ment (intelligence) at 3 yrs							
Dahl et al. 1995 (MHPCD)				Sleep disruption at 3 yrs					
Richardson et al. 1995 (MHPCD)									Motor function at 8 and 18 mos
Chandler et al. 1996 (MHPCD)		Cognitive function at 8 and 18 mos							Gross motor development at 3 yrs
Faden and Graubard 2000						Behavioral problems at 3 yrs			
Noland et al. 2005	Selective attention at 4 yrs								
El Marroun et al. 2011 (Generation R)	Attention problems at 18 mos					Aggressive behavior at 18 mos	Anxiety and/or depression symptoms at 18 mos		
Chakraborty et al. 2015					Global motion perception 4.5 yrs				
Eiden et al. 2018a						Emotional regulation (resp sinus arrhythmia)			
Eiden et al. 2018b						Child behavior problems at 2-3 yrs			
Godleski et al. 2018						Externalizing behavior problems in toddlers			

	ATTENTION	INTELLIGENCE	NEURO-IMAGING	CNS MATURATION	VISUAL-SPATIAL PROCESSING	BEHAVIOR	PSYCHIATRIC SYMPTOMS	SUBSTANCE USE	OTHER
CHILDHOOD (6-12 YRS)									
Tansley et al. 1986 (OPPS Cohort)				Left and right eye, and binocular stimulation					
O'Connell and Fried 1991 (OPPS Cohort)	Distractibility at 6-9 yrs	Comprehension, intelligence at 6-9 yrs			Visual-spatial perception at 6-9 yrs	Parent ratings of behavior problems at 6-9 yrs			
Fried et al. 1992a (OPPS)	Attentional behavior including impulsivity at 6 yrs								
Fried et al. 1992b (OPPS)		Cognitive and receptive language development at 5 and 6 yrs							
Fried et al. 1997 (OPPS)		Reading and language at 9-12 yrs							
Fried et al. 1998 (OPPS)	Cognitive and executive function including attention at 9-12 yrs	Cognitive and executive function including intelligence at 9-12 yrs							
Leech et al. 1999 (MHPCD)	Attention and impulsivity at 6 yrs								
Fried and Watkinson 2000 (OPPS)		Intelligence at 9-12 yrs			Visual-spatial performance at 9-12 yrs				
Goldschmidt et al. 2000 (MHPCD)	Attention problems at 10 yrs								
Richardson et al. 2002 (MHPCD)	Attention and general mental efficiency including impulsivity at 10 yrs	Intelligence and memory at 10 yrs							
Goldschmidt et al. 2004 (MHPCD)		Academic achievement at 10 yrs							
Gray et al. 2005 (MHPCD)							Depressive symptoms at 10 yrs		
Leech et al. 2006 (MHPCD)							Depression and anxiety at 10 yrs		

	ATTENTION	INTELLIGENCE	NEURO-IMAGING	CNS MATURATION	VISUAL-SPATIAL PROCESSING	BEHAVIOR	PSYCHIATRIC SYMPTOMS	SUBSTANCE USE	OTHER
Goldschmidt et al. 2008 (MHPCD)		Intelligence at 6 yrs							
Zammit et al. 2009							Psychotic symptoms at 12 yrs		
Stone et al. 2010									Sleep problems at 12 yrs
Day et al. 2011 (MHPCD)	Attention at 10 yrs					Delinquent behavior at 14 yrs	Depressive symptoms at 10 yrs		
Goldschmidt et al. 2012 (MHPCD)	Attention at 10 yrs	School achievement at 14 yrs					Depressive symptoms at 10 yrs	Substance use	
El Marroun et al. 2016 (Generation R)			Brain volume at 6-8 yrs						
Bolhuis et al. 2018 (Generation R)							Psychotic-like experiences at 10 yrs		
El Marroun et al. 2018 (Generation R)						Behavior and emotional functioning at 7-9 yrs			
ADOLESCENCE (13-18 YRS)									
Fried and Watkinson 2001 (OPPS)	Attention at 13-16 years								
Fried et al. 2003 (OPPS)		Cognitive functioning at 13-16 yrs							
Day et al. 2006 (MHPCD)								Cnb use at 14 yrs	
Porath and Fried 2005 (OPPS)								Cigarette and Cnb use btwn 16-22 yrs	
Rivkin et al. 2008			Brain volume at 10-14 yrs						
Willford et al. 2010a (MHPCD)					Processing speed, visual-motor coordination, interhemispheric transfer at 16 yrs				
Day et al. 2014 (MHPCD)								Early age of Cnb use (< 15 yrs)	

	ATTENTION	INTELLIGENCE	NEURO-IMAGING	CNS MATURATION	VISUAL-SPATIAL PROCESSING	BEHAVIOR	PSYCHIATRIC SYMPTOMS	SUBSTANCE USE	OTHER
Frank et al. 2014								Problematic substance use at 12-18 yrs	
Mathews et al. 2014									Tourette syndrome or chronic tic disorder at 13-14 yrs
De Genna et al. 2015 (MHPCD)						Early vaginal intercourse (<14 yrs), early oral sex (<14 yrs)			
Liebschutz et al. 2015						Behavioral resilience, lack of early substance use or delinquency			
Cornelius et al. 2016 (MHPCD)								Alcohol use at 16 yrs	
Rose-Jacobs et al. 2017	Ability to shift attention between different tasks	Intelligence							
ADULTHOOD (>18 YEARS)									
Smith et al. 2004 (OPPS)	Response inhibition in young adults								
Smith et al. 2006 (OPPS)					Visual spatial working memory in young adults				
Willford et al. 2010b (MHPCD)			Caudate volume asymmetry at 18-22 yrs						
Sonon et al. 2015 (MHPCD)								Frequency of Cnb use at 22 yrs	
Goldschmidt et al. 2016 (MHPCD)						Adult roles at 22 yrs, lifetime conduct disorder at 16 yrs		Early Cnb initiation, behavior at 3 yrs	
Smith et al. 2016 (OPPS)			Visual-spatial processing btwn 18-22 yrs						
Sonon et al. 2016 (MHPCD)							Depressive symptoms at early age (as a mediator)	Early Cnb initiation (as a mediator) Cnb use disorder in young adulthood	

	ATTENTION	INTELLIGENCE	NEURO-IMAGING	CNS MATURATION	VISUAL-SPATIAL PROCESSING	BEHAVIOR	PSYCHIATRIC SYMPTOMS	SUBSTANCE USE	OTHER
De Genna et al. 2018a (MHPCD)								Adult electronic cigarette use	
De Genna et al. 2018b (MHPCD)								Adult co-use of tobacco and Cnb	

Table D.14. Neurodevelopment: Summary of Selected Study Design and Exposure Elements by Age

Study				Exposure			
Author, Year Cohort	**Design**	**Location**	**Outcomes/ Sample size**	**Timing**	**Assessment**	**Quantification**	**Covariates**
Infancy							
Fried 1980 OPPS	Prospective	Ottawa, Canada	Visual response, habituation, tremors, startles, auditory responses, self-quieting, responsiveness (BNBAS) n = 89	1 yr prior to preg and each trimester	Self-report	Categorized as: no use or irregular (≤1 joint/wk or secondhand Cnb exposure); moderate (2-5 joints/wk); heavy (>5 joints/wk)	Alcohol Tobacco
Fried 1982 OPPS	Prospective	Ottawa, Canada	Visual stimuli responsiveness, tremors, startles, habituation, self-quieting, responsiveness, general activity, alertness (BNBAS, BSID) n = 7	1 yr prior to preg and each trimester	Self-report	See Fried 1980	Alcohol Tobacco
Tennes et al. 1985	Prospective	Denver, CO	Neurodevelopment (BNBAS), muscle tone n = 756	During preg and each trimester	Self-report	Categorized as: no use; light (one time only–once/wk); moderate (>once/wk, <daily); heavy (≥daily)	Alcohol Amphetamines Cocaine Tobacco
Fried and Makin 1987 OPPS	Prospective	Ottawa, Canada	Startles, response to light, habituation, tremors, irritability (BNBAS) n = 250	During preg	Self-report	Assessed joints/wk Exposure analyzed as a binary variable	Alcohol Tobacco
Fried et al. 1987 OPPS	Prospective	Ottawa, Canada	Infant state, resting posture, movements, and reflexes at 9 and 30 days (Prechtl) n = 254	1 yr before preg and each trimester	Self-report by interview	Categorized as no/passive exposure or infrequent use (≤1 joint/wk) and regular use (>1 joint/wk)	Alcohol Tobacco
Hayes et al. 1988	Prospective	Jamaica	Neurodevelopment (BNBAS) n = 56	During preg	Self-report by interview Direct observation	Categorized as: no use; irregular; moderate; heavy Exposure assessed as binary for BNBAS analysis	Study noted use of alcohol and tobacco was infrequent in population
Scher et al. 1988 MHPCD	Prospective	Pittsburgh, PA	Disturbance in neonatal sleep cycling n = 55	1 yr prior to preg and each trimester	Self-report	Average joints/day Categorized as users if used Cnb at least once/day, otherwise non-users	Alcohol Tobacco
Richardson et al 1989 MHPCD	Prospective	Pittsburgh, PA	Neurodevelopment (BNBAS)	Each trimester	Self-report by interview	Average joints/day for each trimester Categorized as no use; <1/mo; 1-3/mo; 1-6/wk; ≥/day	Alcohol Tobacco Other illicit drugs
Parker et al. 1990	Prospective	Boston, MA	Jitteriness n = 1,054	During preg	Urine Self-report	Exposure assessed as binary based on self-report and urine separately	No adjusted analysis
Scher et al. 1998 MHPCD	Prospective	Pittsburgh, PA	Visually evoked potentials at 1, 4, 8, and 18 mos n = 74	1 yr prior to preg and each trimester	Self-report	Categorized as no use; light (>0-3 joints/wk;	Alcohol Other drugs Tobacco

Study				Exposure			
Author, Year Cohort	**Design**	**Location**	**Outcomes/ Sample size**	**Timing**	**Assessment**	**Quantification**	**Covariates**
						moderate (4-6 joints/wk); heavy (≥1 joint/day)	
De Moraes Barros et al. 2006	Prospective	Sao Paulo, Brazil	Neurodevelopment (NNNS) n = 561	During preg	Self-report Maternal hair Meconium	Exposure assessment binary based on biological assay	Alcohol Other drugs Tobacco
Stroud et al. 2018	Prospective cohort	Providence, RI	Self-regulation, handling, lethargy, attention (NNNS) n = 111	Each trimester	Self-report	Exposure assessed as days of use Analyzed as binary bariable	Tobacco Other substance use
Early childhood (1-5 yrs)							
Fried and Watkinson 1988 OPPS	Prospective	Ottawa, Canada	Mental and language development at 1 and 2 yrs, motor development at 2 yrs n = 217 at 1 yr; 157 at 2 yrs	During preg	Self-report	Joints/wk Categorized as no use or heavy use (>5 joints/wk)	Alcohol Tobacco
Fried and Watkinson 1990 OPPS	Prospective	Ottawa, Canada	Cognitive functioning at 3 and 4 yrs (motor, language, memory) n = 130 at 3 yrs; 123 at 4 yrs	During preg	Self-report	Categorized as infrequent/ no use (<1 joint/wk); moderate, (>1 joint/wk - <6 joints/wk); heavy (≥6 joints/wk)	Alcohol Tobacco
Day et al. 1994b MHPCD	Prospective	Pittsburgh, PA	Cognitive development and intelligence at 3 yrs n = 655	Each trimester	Self-report	Average joints/day	Alcohol Other drugs Tobacco
Dahl et al. 1995 MHPCD	Prospective	Pittsburgh, PA	Sleep disruption at 3 yrs n = 38	Each trimester	Self-report	Exposure assessed during 1st trimester Exposed if <1 joint/mo, otherwise unexposed	Alcohol Other drugs Tobacco
Richardson et al. 1995 MHPCD	Prospective	Pittsburgh, PA	Motor function at 8 and 18 mos n = 569 at 18 mos	Each trimester	Self-report	Average joints/day Categorized as: no use; Light (0<joints/day to <0.4 joints/day) moderate(≤0.4 joints/day to 1 joint/day); heavy (≥1 joint/day) Exposure analyzed as binary variable for each trimester	Alcohol Other drugs Tobacco Current maternal substance use
Chandler et al. 1996 MHPCD	Prospective	Pittsburgh, PA	Cognitive function at 8 and 18 mo; Gross motor development at 3 yrs n = 650	1 yr prior to preg and each trimester	Self-report	Average joints/day Categorized as no use; light/moderate (0-1 joint/day) heavy (≥1 joint/day) Exposure analyzed as binary variable for each trimester	Alcohol Other drugs Tobacco
Faden and Graubard 2000		US	Behavioral problems at 3 yrs	Before and during preg	Self-report	No drug use (0) < 1 use/month (0.5) 1 use/month (1) 2-3 uses/month (2.5) 1-2 ues/week (6) >3 ues/week (14)	
Noland et al. 2005	Prospective	Cleveland, OH	Selective attention at 4 yrs n = 330	Each trimester	Meconium Infant and maternal	Classified as exposed if positive self-report, urine, or meconium Also classified by average joint/day	Cocaine Current substance use

Study				Exposure			
Author, Year Cohort	**Design**	**Location**	**Outcomes/ Sample size**	**Timing**	**Assessment**	**Quantification**	**Covariates**
					urine Self-report		
El Marroun et al. 2011 Generation R	Prospective	Rotterdam, the Netherlands	Attention problems, aggressive behavior, and anxiety and/or depression symptoms at 18 mos n = 4,077	3 mos prior to preg and during preg	Self-report	Categorized as: never use; use before preg; use up to knowledge of preg; continued use through preg Quantified as daily, weekly, or monthly	Alcohol Other drugs Tobacco
Chakraborty et al. 2015	Prospective	Auckland, New Zealand	Global motion perception at 4.5 yrs n = 165	During preg	Self-report Meconium	Categorized as light (<1 joint/occasion); moderate (1-2 joints/occasion); heavy (>2 joints/ occasion)	Alcohol Other drug use
Eiden et al. 2018a	Prospective	Buffalo, NY	Emotional regulation in toddlers based on respiratory sinus arrhythmia n = 247	Each trimester During preg	Self-report Maternal and infant saliva Meconium	Average joints/day Exposure analyzed as binary variable	
Eiden et 2018b	Prospective	Buffalo, NY	Child behavior problems at 2-3 yrs n = 232 at 2 yrs; 206 at 3 yrs	Each trimester During preg	Meconium Maternal saliva Self-report by interview	Average joints/day Exposure analyzed as binary variable based on self-report, meconium, or saliva	
Godleski et al. 2018	Prospective	Pittsburgh, PA	Externalizing behavior problems in toddlers	During preg	Meconium Saliva Self-report by interview	Exposure assessed as binary variable based on self-report or biological assay	
Childhood (6-12 yrs)							
Tansley et al. 1986 OPPS	Prospective	Ottawa, Canada	Left and right eye stimulation, binocular stimulation btwn 3-10 yrs (CNS Maturation) n = 101	During preg	Urine toxicology Self-report	Exposure analyzed as binary variable based on urine or self-report	Alcohol Tobacco
O'Connell and Fried 1991 OPPS	Prospective	Ottawa, Canada	Distractibility, comprehension, intelligence, visual-spatial perception, behavior problems at 6-9 yrs n = 56	During preg	Self-report	Cnb use measured in joints/wk Use > 1 joint/wk considered regular use	Miscellaneous variables listed in summary
Fried et al. 1992a OPPS	Prospective	Ottawa, Canada	Attentional behavior including impulsivity at 6 yrs n = 126	During preg	Self-report	Categorized as: no/ infrequent use; moderate use (>1 joint/wk, <6 joints/wk); heavy use (≥6 joints/wk)	Alcohol Tobacco
Fried et al. 1992b OPPS	Prospective	Ottawa, Canada	Cognitive and receptive language development at 5 and 6 yrs n = 135 at 5 yrs; 137 at 6 yrs	During preg	Self-report	See Fried et al. 1992a	Cnb not included in multivariable analysis
Fried et al. 1997 OPPS	Prospective	Ottawa, Canada	Reading and language at 9-12 yrs n = 131	During preg	Self-report	See Fried et al. 1992a	Alcohol Other drugs Tobacco
Fried et al. 1998 OPPS	Prospective	Ottawa, Canada	Cognitive and executive function including attention and intelligence at 9-12 yrs n = 131	During preg	Self-report	See Fried et al. 1992a	Alcohol Other drugs Tobacco Current maternal Cnb

Study				Exposure			
Author, Year Cohort	**Design**	**Location**	**Outcomes/ Sample size**	**Timing**	**Assessment**	**Quantification**	**Covariates**
Leech et al. 1999 MHPCD	Prospective	Pittsburgh, PA	Attention and impulsivity at 6 yrs n = 608	Each trimester	Self-report	Average joints/day Analyzed as binary variable for each trimester	Alcohol Cocaine Tobacco Current maternal Cnb
Fried and Watkinson 2000 OPPS	Prospective	Ottawa, Canada	Intelligence and visual-spatial performance at 9-12 yrs n = 146	During preg	Self-report	See Fried et al. 1992a	Other drugs Tobacco Postnatal passive smoke exposure
Goldschmidt et al. 2000 MHPCD	Prospective	Pittsburgh, PA	Attention problems at 10 yrs n = 635 with mothers' report; 575 with teachers' report	Each trimester	Self-report	Average joints/day Categorized as no use; light/moderate (0-0.89 joints/day); heavy (>0.89 joints;day) Exposure assessed as binary by trimester for some analysis, and by category for others	Alcohol Cocaine Tobacco Current substance use
Richardson et al. 2002 MHPCD	Prospective	Pittsburgh, PA	Neurophysiological outcomes including attention and intelligence at 10 yrs n = 593	Each trimester	Self-report	See Richardson et al 1995	Alcohol Tobacco Current substance use
Goldschmidt et al. 2004 MHPCD	Prospective	Pittsburgh, PA	Academic achievement at 10 yrs n = 606	Each trimester and during preg	Self-report	Categorized by ADJ as: no use; light/moderate (< joint/day); heavy (≥1 joint/day) Exposure analyzed as binary per trimester	Other prenatal substance use
Gray et al. 2005 MHPCD	Prospective	Pittsburgh, PA	Depressive symptoms at 10 yrs	Each trimester	Self-report	See Richardson et al 1995	Current maternal substance use
Leech et al. 2006 MHPCD	Prospective cohort	Pittsburgh, PA	Depression and anxiety at 10 yrs n = 636	During preg	Self-report	Average joints/day Categorized as users (≥2 joints/mo), and non-users (<2 joints/mo)	Tobacco
Goldschmidt et al. 2008 MHPCD	Prospective	Pittsburgh, PA	Intelligence at 6 yrs n = 648	Each trimester	Self-report	Average joints/day Categorized as: no use; light/moderate (<1 joint/day); heavy (≥1 joint/day)	Alcohol Other drugs Tobacco Current substance exposure
Zammit et al. 2009	Prospective	Avon, UK	Psychotic symptoms at 12 yrs n = 150	During preg and each trimester	Self-report	Categorized by frequency as no use, <weekly, ≥weekly	
Stone et al. 2010	Prospective	RI, FL, MI, TN	Sleep problems at 12 yrs n = 808	During preg	Self-report by interview	Number of joints during pregnancy	
Day et al. 2011 MHPCD	Prospective	Pittsburgh, PA	Attention and depressive symptoms at 10 yrs, delinquent behavior at 14 yrs n = 525	During preg	Self-report by interview	Average joints/day Exposure analyzed as binary variable	First trimester alcohol and tobacco use
Goldschmidt et al. 2012 MHPCD	Prospective	Pittsburgh, PA	Attention and depressive symptoms at 10 yrs, school achievement at 14 yrs, adolescent substance use n = 524	Each trimester	Self-report by interview	Average joints/day Categorized as: no use; heavy (≥1 joint/day) non-heavy (<1 joint/day)	Alcohol Other drugs Tobacco Current maternal use

Study				Exposure			
Author, Year Cohort	**Design**	**Location**	**Outcomes/ Sample size**	**Timing**	**Assessment**	**Quantification**	**Covariates**
El Marroun et al. 2016 Generation R	Prospective	Rotterdam, the Netherlands	Brain volume at 6-8 yrs n = 263	During preg	Urine Self-report	Exposure assessed as binary based on self-report or urine	Alcohol
Bolhuis et al. 2018 Generation R	Prospective	Rotterdam, the Netherlands	Psychotic-like experiences at 10 yrs n = 7,393	During preg	Self-report Urine	Categorized as: no use; before preg: during preg	Alcohol Tobacco
El Marroun et al. 2018 Generation R	Prospective	Rotterdam, the Netherlands	Behavior and emotional functioning at 7-9 yrs n = 5,903	Before or during preg	Self-report Urine	Categorized as: no use; before preg during preg	Alcohol Tobacco
Adolescence (13-18 yrs)							
Fried and Watkinson 2001 OPPS	Prospective	Ottawa, Canada	Attention at 13-16 yrs n = 152	During preg	Self-report	See Fried et al. 1992a	Alcohol Tobacco Current maternal drugs
Fried et al. 2003 OPPS	Prospective	Ottawa, Canada	Cognitive functioning at 13-16 yrs n = 145	During preg	Self-report	Categorized as: no / moderate use (<6 joints/wk); heavy use (≥6 joints/wk)	Alcohol Other drugs Tobacco Current maternal Cnb
Day et al. 2006 MHPCD	Prospective	Pittsburgh, PA	Cnb use at 14 yrs n = 563	During preg and each trimester	Self-report	Average joints/day	Family history of alcohol or drug problems
Porath and Fried 2005 OPPS	Prospective	Ottawa, Canada	Cigarette and Cnb use btwn 16-22 yrs n = 152	During preg	Self-report	Exposure analyzed as binary for analysis	Alcohol Tobacco
Rivkin et al. 2008	Prospective cohort	Boston, MA	Brain volume at 10-14 yrs n = 35	During preg	Self-report Meconium	Use during preg was binary based on self-report or meconium	Alcohol Cocaine Tobacco
Willford et al. 2010a MHPCD	Prospective	Pittsburgh, PA	Processing speed, visual-motor coordination, interhemispheric transfer at 16 yrs n = 320	Each trimester	Self-report by interview	Average joints/day Categorized as: no use; light/moderate (<1 joint/day); heavy (≥1 joint/day)	Alcohol Tobacco Current maternal substance use
Day et al. 2015 MHPCD	Prospective	Pittsburgh, PA	Early age of Cnb use (<15 yrs) n = 596	During preg	Self-report by interview	Assessed and analyzed as ADJ	Alcohol Tobacco
Frank et al. 2014	Prospective	Boston, MA	Problematic substance use 12-18 yrs n =157	During preg	Self-report Urine Meconium	Categorized by frequency as no use, heavy use (positive meconium or urine assay during gestation or delivery, or >8 days self-reported use during preg), or light use (positive by self-report or urine, but not meeting criteria for heavy use)	Alcohol Tobacco
Mathews et al. 2014	Prospective	Avon, UK	Tourette syndrome or chronic tic disorder at 13-14 yrs n = 6,090	Prior to preg, 1st trimester, and last 2 mos preg	Self-report by questionnaire	Exposure assessment was binary	Alcohol in last 2 mos preg Tobacco in last 2 mos preg

Study				Exposure			
Author, Year Cohort	**Design**	**Location**	**Outcomes/ Sample size**	**Timing**	**Assessment**	**Quantification**	**Covariates**
De Genna et al. 2015 MHPCD	Prospective	Pittsburgh, PA	Early vaginal intercourse, early oral sex (<14 yrs) n = 324	During preg	Self-report by questionnaire	Exposure assessed as ADJ, dichotomized for analysis	
Liebschutz et al. 2015	Prospective	Boston, MA	Behavioral resilience: no early substance use <14 yrs, no risky sexual behavior, no delinquency; n = 136	During preg	Self-report Urine Meconium	Exposure analyzed as binary variable based on self-report, urine, or meconium	
Cornelius et al. 2016 MHPCD	Prospective	Pittsburgh, PA	Alcohol use at 16 yrs n = 917	2nd trimester only	Self-report	Average joints/day	
Rose-Jacobs et al. 2017	Prospective	Boston, MA	Ability to shift attention between tasks, intelligence in high school students n = 131	During preg	Self-report Urine Meconium	Exposure based on urine, meconium, or maternal self-report	Other prenatal substance exposure
Adulthood (>18 yrs)							
Smith et al. 2004 OPPS	Prospective	Ottawa, Canada	Response inhibition in young adults n = 31	During preg	Self-report	Cnb measured in average weekly joints Considered exposed if used >1 joint/wk	Alcohol Tobacco Current maternal substance use
Smith et al. 2006 OPPS	Prospective	Ottawa, Canada	Visual spatial working memory in young adults n = 31	During preg	Self-report	See Smith et al. 2004	Alcohol, Tobacco Current maternal substance use
Willford et al. 2010b MHPCD	Prospective	Pittsburgh, PA	Caudate volume asymmetry at 18-22 yrs n = 45	Prior to preg and 1st trimester	Self-report	Average joints/day Categorized as: no use; moderate (0-1 joint/day); heavy (≥joint/day)	Alcohol Tobacco
Sonon et al. 2015 MHPCD	Prospective	Pittsburgh, PA	Frequency of Cnb use at 22 yrs n = 763	Each trimester	Self-report by interview	Average joints/day, categorized by frequency as light to moderate (<1 joint/wk), and heavy (≥1 joint/week)	Alcohol
Goldschmidt et al. 2016 MHPCD	Prospective	Pittsburgh, PA	Adult roles at 22 yrs, lifetime conduct disorder at 16 yrs n = 608	Each trimester	Self-report by interview	See Richardson et al. 1989	Alcohol Tobacco
Smith et al. 2016 OPPS	Prospective	Ottawa, Canada	Visual-spatial processing btwn 18-22 yrs n = 31	During preg	Self-report	See Smith et al. 2004	Alcohol Tobacco
Sonon et al. 2016 MHPCD	Prospective	Pittsburgh, PA	Depressive symptoms at an early age, early age of Cnb initiation, Cnb use disorder in young adulthood n = 590	During preg	Self-report	Average joints/day Exposure assessed as binary variable	Alcohol in the 1st trimester
De Genna et al. 2018a MHPCD	Prospective	Pittsburgh, PA	Adult electronic cigarette use n = 427	During preg	Self-report	Average joints/day Exposure assessed as binary variable	Alcohol
De Genna et al. 2018b MHPCD	Prospective	Pittsburgh, PA	Adult co-use of tobacco and Cnb	During preg	Self-report	Average joints/day Exposure assessed as binary variable	Alcohol, Substance use disorders

Tests used to assess neurodevelopmental effects in cannabis epidemiological studies

Epidemiological studies of neurodevelopment relied on a variety of tests. This section, adapted from Fried et al. (1998), gives brief explanations of the various tests used, organized alphabetically by the name of the test.

Abstract Designs: The subject is directed to touch 1 out of 12 abstract designs on the first 12 pages and a different design on each successive page without touching any design multiple times. Both latency and errors are collected as measures (Fried et al. 2003).

Auditory Working Memory: This test measures the participant's ability to retain information in short-term memory while processing incoming information and retrieving information from long-term storage. "The participant was instructed to read aloud blocks of 2, 3, 4, or 5 sentences and asked to fill in a missing word at the end of each sentence within a block." The participant then had to remember the missing words within each block. (Fried et al. 1998).

2-Back Test: This test requires participants to press a button every time an "O" is presented in the same position that it was in 2 presentations before (Smith et al. 2006).

Bayley Scales of Infant Development (BSID): This test consists of three scales including: the mental developmental index, the psychomotor developmental index, and the infant behavior record. The mental developmental index assesses sensory perceptual abilities, the psychomotor developmental index assesses gross and fine motor movement, and finally the infant behavior record evaluates the infant's attitudes, interests and temperament (Fried and Watkinson 1988).

Bogus Pipeline Method: Participants were led to believe that interviewers would confirm their substance use reports with biological testing, although not true (Day et al. 1985).

Brazelton Neonatal Behavioral Assessment Scale (BNBAS): This assessment evaluates the infant's responsiveness to the interaction with the examiner, their capacity for self-organization, and individual differences in neonatal behavior. The tester observes and scores various infant responses including: response decrement to visual, auditory, and tactile stimuli; irritability, self-quieting, and consolability; visual and auditory responsiveness to inanimate and animate stimuli, motor maturity; and primitive reflexes (Richardson et al. 1989).

Category Test: This test measures concept formation requiring non-verbal abstract reasoning and mental flexibility. The test involves a projector presenting 208 images consisting of 7 subsets. The participant is given a console with 4 colored buttons. The participant is "required to abstract principles in each subtest based on variables of size, shape, number and position of objects and press the appropriate response button." A bell was used to provide immediate positive feedback and a buzzer provided immediate negative feedback. The total number of errors is often the reported measure (Fried et al. 1998).

Child Behavior Checklist: This is a standardized maternal report of children's problem behaviors. The checklist includes a variety of syndrome scales such as Anxious/Depressed, Attention Problems and Aggressive Behavior etc. Each item is rated on a scale ranging from 0 (not true), 1 (somewhat or sometimes true), and 2 (very true or often true). The questions specifically ask about behavior of the child in the past two months (El Marroun et al. 2011).

Children's Depression Inventory (CDI): This test measures general psychopathology and distress (Goldschmidt et al. 2012).

Conners Parent Questionnaire: This test is a measure of a child's behavior based upon parental assessment. The test evaluates 48 behavioral items which are given scores of "not at all", "just a little", "pretty much", or "very much". The test yields 6 z scores on the following: Conduct Problems, Learning

Problems, Psychosomatic Problems, Impulsivity-Hyperactivity, Anxiety, and Hyperactivity Index (O'Connell and Fried 1991).

Continuous Performance Test (CPT): This test assesses impulsivity, sustained attention, and general mental efficiency. The participant is presented with a series of colored letters and the participant responds to a target stimulus when it is preceded by a specific letter. The nature of the symbols can vary based upon the edition of CPT used, for example it may present varying shapes instead of letters. Errors of omission and commission are typically collected. Many variations of this test exist (Richardson et al. 2002 and Leech et al. 1999).

Fluency Test: This test measures oral fluency in the participant. The participant was expected to produce as many words as possible beginning with a specific consonant sound within 60 seconds (Fried et al. 1998).

fMRI: This noninvasive neuroimaging technique provides a measure of neural activity while a participant is performing a task. As neural activity occurs, the regional cerebral blood flow increases while the deoxyhemoglobin contribution decreases resulting in a measurable change in magnetic signal (Smith et al. 2006).

Go/No-Go: This test is used to assess response inhibition. A participant is required to initiate a motor response (i.e. press a button) when exposed to a particular stimulus and withhold a response when shown a different stimulus (No Go) (Smith et al. 2004).

Gordon Delay Task: This test measures impulsivity and is a specific kind of CPT. The participant is given a one-button solid state console and a six second differential reinforcement of low rate responding schedule. The participant receives points on a screen, "reinforcements", when a button press occurs six seconds after a previous button press. The total number of responses, the number of correct responses, and the efficiency ratio are typically reported (Fried et al. 1998).

Gordon Vigilance Task: This test measures sustained attention and is a specific kind of CPT. The same apparatus used in a "Gordon Delay Task" are used here. A series of single-digit numbers are shown for 200 milliseconds every 1 second over the course of 9 minutes. The participant is required to press a response button when a target stimulus appears. The total correct responses and number of commission errors are typically reported. (Fried et al. 1998)

The Home Environment Questionnaire (HEQ): This questionnaire evaluates the environment a child is raised in and includes 10 scales: Achievement, Aggression-External, Aggression in the Home, Aggression Total, Supervision, Change, Affiliation, Separation, Sociability, and Socioeconomic Status (Fried et al. 1998).

*The HOME Inventor*y: This test collects information by observation of the child's home environment and by questions addressed to the mother. The test assesses six categories: emotional and verbal responsivity of the mother, avoidance of restriction and punishment, organization of the environment, provision of appropriate play materials, maternal involvement and opportunities for variety in daily routine (Fried and Watkinson 1988).

Knox Cube Test: The participant is given a row of four cubes affixed on a strip of wood. The participant "is required to imitate prearranged sequences of increasing length and complexity as touched at a rate of 1/s" (Fried et al. 2003).

Match to Centre: The participant is required to press a button each time an O is presented in the middle of the screen (as opposed to any other position on the screen) (Smith et al. 2006).

McCarthy Scales of Children's Abilities: This test contains six scales: verbal, perceptual, quantitative, memory, motor, and a general cognitive composite scale that combines verbal, perceptual and quantitative into one (Fried and Watkinson 1990).

Missing Numbers The participant must state which number is missing when numbers from 1-10 are read in random sequence with one number omitted (Fried et al. 2003).

The NEO Five Factor Inventory: This test is used to assess the mother's personality. It provides measures of Neuroticism, Extraversion, Openness, Agreeableness, and Conscientiousness (Fried et al. 1998).

*Neonatal Intensive Care Unit Network Neurobehavioral Scale (NNNS):*This test "evaluates neurologic integrity, behavioral function, and the presence of stress and abstinence signs from the neonate…" The NNNS includes "13 variables including habituation, attention, arousal, regulation, handling, quality of movement, excitability, lethargy, nonoptimal reflexes, asymmetry, hypertonicity, hypotonicity, and stress/abstinence signals" (De Moraes Barros 2006).

Peabody Individual Achievement Test (PIAT): Spelling Recognition Subtest- the participant is orally presented a word and is required to visually select the correct spelling of a word out of several slightly different written variations (Fried et al. 2003).

Peabody Picture Vocabulary Test: This test measures receptive vocabulary (Fried and Watkinson 1990). It assesses auditory comprehension of picture names (Fried and Watkinson 1997).

Pegboard Test: This test measures speed and accuracy of the participant's eye-hand coordination. The participant is instructed to place keyhole-shaped metal pegs into rows of matching holes in a board as quickly as possible (Fried and Watkinson 1990).

Picture Deletion Task: This is a visual search task in which the participant is instructed to find target pictures among foils. The numbers of each might vary per study. For example, in Noland et al. 2005 the participants were directed to find 30 target pictures among 90 foils. Both the target pictures and foils are presented at once on two pieces of paper (Noland et al. 2005).

The Reynell Developmental Language Scales: This test measures comprehension and expression (Fried and Watkinson 1988).

Sentence Memory Test: The participant must repeat 25 sentences of increasing length and complexity (Fried et al. 2003).

Stanford-Binet Intelligence Scale (SBIS): A measure of cognitive development and intelligence. The test contains four subtests including: verbal reasoning, quantitative reasoning, abstract/visual reasoning, and short-term memory. The verbal and quantitative reasoning subareas indicate crystallized intelligence or scholastic abilities because they represent skills learned via education and acquired knowledge. Otherwise, the abstract/visual reasoning subtest assesses the fluid-analytic ability and requires more cognitive skills to solve nonverbal tasks (Goldschmidt et al. 2008).

Stroop Test: The participant is required to read the words "red", "green", and "blue" printed repeatedly in black ink. They are then required to name the colors of stimuli "XXXX" written repeatedly in red, green, or blue ink. Finally, they are required to name the colors in which the words "red", "green", and "blue" are written repeatedly as the color of the words do not match the color they are printed in (Fried et al. 2003).

The Swanson, Noland, and Pelham (SNAP) attention subscale consists of 5 questions based on the DSM-III definition of attention deficit disorder. Answers range from never to all the time and assesses symptoms such as "easily distracted" or "failing to finish things" (Goldschmidt et al. 2012).

Tactile Form Recognition Task: The participant is required to identify flat plastic shapes placed in their hand while they cannot see it. The child identifies the shape by matching it to one of four different stimulus shapes that they are shown (Fried and Watkinson 1990).

Tactual Performance Task: The participant must place wooden blocks of varying shapes into their slot on a foam board while blindfolded. This task is done first with the dominant hand, then with the non-

dominant hand, and finally with both hands. The length of time taken to complete the task is the main measure of performance (Fried et al. 1998).

Test of Visual Perceptual Skills: This test evaluates a participant's capabilities in several categories including:

- Visual discrimination- the participant must select an exact match from an array of similar forms
- Visual memory- the participant must remember the characteristics of a stimulus by finding it in an array of similar stimuli. This is a test of immediate recall.
- Visual-Spatial relationships- the participant must select the identical form among many similar forms. The original form will be in a different orientation.
- Visual form constancy- the participant must again find a particular stimulus identical to what had been presented previously. The stimulus will be smaller, larger, rotated, and/or hidden
- Visual sequential memory- the participant must remember for immediate recall sets of forms among different arrays of similar sets of forms
- Visual figure-ground- the participant must find a form hidden in a conglomerated ground of matter
- Visual closure- the participant must be able to choose a complete stimulus from incomplete forms of a stimulus presented to them previously.

This test provides an overall Perceptual Quotient based on all the subtests (O'Connell and Fried 1991).

Timeline Follow Back Interview (TLFB): The Timeline follow-back interview yields daily information regarding maternal substance use. The mothers are instructed to identify the approximate date of conception on a calendar along with anchor points to aid recall. These anchor points involved pointing out different events in each month such as birthdays, holidays, parties, sports events, and funerals. Interviews are conducted every trimester and at each interview mothers provide information about substance use for the previous three months. The TLFB provides the average number of cigarettes and joints smoked per day and average number of alcoholic drinks per day throughout the pregnancy. This method of collecting substance use patterns reportedly is reliable and valid, has good test-retest reliability, and has a high correlation with other self-report measures.

Trail Making Test: This test measures attention, visuomotor tracking, and problem solving. In Trails Part A, a participant is shown a sheet of paper with circled numbers arranged randomly across the page. The participant was required to connect the numbers in the correct order as quickly as they could. In Trails Part B, the sheet of paper had both letters and numbers and the participant was required to alternate between the two in sequence as quickly as possible (Richardson et al. 2002).

Visuospatial n Back Task: The participant is instructed to press a button when a specific letter is presented (control condition), and to press when the letter that was presented a certain number of letters previously is shown again. For example, a subject may be asked to press the button when the same letter presented 2 letters ago is presented again (A-B-C-B and the participant must respond to the second B) (Smith et al. 2006.

Wechler Individual Achievement Test (WIAT): This test is often used to measure academic achievement. The Screener is composed of three subsets including: basic reading, mathematics reasoning, and spelling. The basic reading component involves assessing basic decoding letters and words. The mathematics reasoning subtest evaluates problem-solving strategies. The spelling component involves encoding dictated sounds and words (Goldschmidt et al. 2012).

Wide Range Assessment of Memory and Learning (WRAML): This test is used to measure learning and memory through four subtests that examine picture, design, story memory, and verbal learning (Richardson et al. 2002).

Wide Range Achievement Test (WRAT):

- WRAT-R This test is a single word reading recognition subtest. Participants are asked to read written words of increasing difficulty.
- WRAT-Spelling Participants are orally presented increasingly difficult words to be written.
- WRAT-Arithmetic Participants are given arithmetic problems of increasing difficulty. (Fried et al. 2003).

WISC-III: This test of general intellectual capabilities contains 13 subtests: Information, Similarities, Arithmetic, Vocabulary, Comprehension, Digit Span, Picture Completion, Coding, Picture Arrangement, Block Design, Object Assembly, Symbol Search, and Mazes. The test also provides a composite Verbal and Performance IQ, Full Scale IQ, and derived Verbal Comprehension, Perceptual Organization, Freedom from Distractibility and Processing Speed Factor scores (Fried et al. 1998).

- Block Design: This concept formation task requires perceptual organization, spatial visualization and abstract conceptualization. Subjects are shown a picture of blocks and are required to assemble the blocks in a form identical to the picture.
- Picture Completion: This task tests the participant's ability to differentiate essential from nonessential details. Subjects are required to identify a missing portion of an incomplete picture.

Wisconsin Card Sorting Test: This test is used to assess a participant's problem solving and abstract reasoning. It provides a measure of deductive reasoning and a participant's ability to shift to a new strategy. Participants are required to match stimulus cards according to color, shape, or number. When a participant completed 10 successive, correct responses, the matching criteria is changed. The participant then has to deduce the new criteria through feedback they receive (Richardson et al. 2002).

Woodcock Reading Mastery Test: This test assess participant's reading capabilities. The Passage Comprehension subtest requires the subject to read short passages and supply a key word missing from each passage (Fried et al. 1997).

Central Nervous System Maturation

Seven studies assessed maturation of the central nervous system (CNS) in infants and young children exposed prenatally to cannabis (See Appendix Table 2.1). Two of these studies used visual evoked potentials as an indicator of maturation of visual pathways (Tansley et al. 1986 and Scher et al. 1998). Tansley et al. (1986) noted a slight tendency toward longer P1 latencies (though no statistics were reported) and more variability of binocular indices ($p<0.05$) for both the latency and amplitude among cannabis-exposed children ages 3 years and older in the OPPS cohort. Scher et al. (1998) found prolonged latencies of P_1 waveform at 1 and 18 months (but not at 8 months) associated with 3rd trimester cannabis exposure, in the MHPCD cohort. Tansley et al. suggest their results are consistent with delayed central nervous system maturation and Scher et al. (1998) more specifically state the data are consistent with a transient delay in neurophysiologic maturation of the visual system, particularly during infancy, for the P_1 wave.

Two MHPCD studies used EEGs to assess sleep, one in neonates 24-36 hours after birth (Scher et al. 1988) and one in 3-year-olds (Dahl et al. 1995). Scher et al. (1998)

describes EEG sleep patterns and visual evoked potentials as measuring similar neurophysiologic dysfunction. Scher et al. (1988) found that cannabis exposure in each trimester was associated with increased body movements, decreased total quiet sleep, and decreased trace alternant quiet sleep in neonates. Increased mixed active sleep, decreased low voltage irregular active sleep, and fewer rapid eye movements were also associated with prenatal cannabis exposure. Scher et al. (1998) speculate that cannabis exposure may contribute to overelaboration of the noradrenergic system, reflected in increased motility and longer sleep segments. Dahl et al. examined sleep in a related cohort (31 of the 38 participants had been in the neonate study) and reported lower sleep efficiency, more arousals, and more awake time associated with 1st trimester cannabis exposure.

Three OPPS studies examined response and habituation to light in neonates (Fried 1980, Fried and Makin 1987, Fried 1982). Fried (1980) used the Brazelton Neonatal Behavioral Assessment Scale (BNBAS) to assess 2- to 3-day-old neonates and found that infants exposed to cannabis were less likely to respond to light and habituate to light (some of these findings were of marginal statistical significance). Cannabis-exposed infants were also reported to be more apt to exhibit marked tremors and startles. Fried and Makin (1987) also used BNBAS to assess 3- to 6-day-olds and found a reduction in habituation to light, and increased startles, tremors, and irritability after controlling for possible confounders, including cigarettes and alcohol. Responsiveness to light was no longer statistically significantly diminished compared to unexposed infants. Fried (1982) reported that visual stimuli responsiveness, tremors, and startles, were similar to unexposed children by 30 days of age.

Six of the seven studies reported associations between prenatal exposure to cannabis and indicators of altered CNS maturation. There were statistically significant results for BNBAS scores (Fried 1980, Fried and Makin 1987) that appear to have dissipated by one month of age (Fried 1982), visual evoked potentials (Tansley et al. 1987 and Scher et al. 1998), and sleep (Scher et al. 1988 and Dahl et al. 1995). The data are all from two prospective cohorts: the MHPCD cohort comes from a low SES population and the OPPS cohort comes from a low-risk population.

Attention

Overall, 12 prospective studies examined associations between prenatal cannabis use and attention deficits in offspring. Details are provided in Appendix Table 2.2. According to Fried et al (1992a), Attention Deficit Hyperactivity Disorder is an externalizing behavior disorder that involves impulsivity, the inability to sustain attention, and hyperactivity with a typical onset at the age of 4.

Six of the studies examined associations between prenatal cannabis use and attention deficits in offspring using Continuous Performance Tests (CPT). Impulsivity in these

studies is measured in the form of errors of commission, in which the child presses a button when a non-target stimulus is presented, indicating a lack of behavioral inhibition in the child (Fried et al. 1992a). Inhibitory functions are associated with the orbitofrontal regions of the prefrontal cortex and its connections with the amygdala, hypothalamus, medial thalamus, and ventromedial caudate (Fried and Watkinson 2001). Sustained attention was measured through errors of omission or when the child failed to press a response button when the target stimulus was presented (Fried et al. 1992a).

Out of the six studies using CPT, two were conducted with the MHPCD cohort (Leech et al., 1999; Richardson et al. 2002), three with the OPPS cohort (Fried et al., 1992a; Fried et al., 1998; Fried and Watkinson, 2001), and one in Cleveland, OH (Noland et al 2005).

CPT studies were conducted on participants ranging from ages 4-13 years old.

Early Childhood (1-5 yrs):

Noland et al. (2005) evaluated selective attention in 4-year old offspring (preschoolers) using a CPT and a picture deletion task (PDT) and found a non-significant positive correlation between average severity of prenatal cannabis exposure and rate of omission errors on the PDT. After controlling for prenatal cocaine exposure and current caregiver cannabis use, the association was decreased substantially. This study lacked a cannabis-only exposure group. Although prenatal exposure to cocaine was statistically controlled for, it is unclear whether prenatal alcohol and tobacco exposures were controlled for as well.

Childhood (6-12 yrs)

Age 6 years

Two studies looked at attention in 6-year old children (Fried et al 1992a, Leech et al., 1999). The first study by Fried et al (1992a), conducted in the OPPS cohort, measured attentional behavior using a battery of tests including the Gordon Diagnostic System Vigilance Task (a version of CPT) and the Conner's Impulsive-Hyperactive scale of CPRS-48. The results showed prenatal cannabis exposure was significantly positively associated with the number of omissions on the CPT, total correct on the CPT, as well as scores of the impulsive/hyperactive scale of CPRS, indicating that individuals with prenatal cannabis exposure were more impulsive/hyperactive according to the scale. Additionally, the number of omission errors and number of correct answers were shown to have a dose-response relationship with prenatal cannabis exposure where children with heavier exposure committed more errors than those with lower exposure. The results of this study indicate weakened sustained attention and higher impulsivity.

In the study conducted in the MHPD Cohort (Leech et al. 1999), the researchers observed a statistically significant increase in errors of commission in CPT tasks after controlling for postnatal cannabis exposure and other covariates. Interestingly, this study

also found lower errors in omission indicating increased attentiveness in children, contrary to what was shown in other studies. The authors theorize that this may be because children were slowing down responses in order to achieve accuracy. The authors note that “if increased accuracy is achieved at the cost of processing speed, the children may do less well over the long-term, particularly in time limited situations”. However, since the study did not collect reaction times they were unable to test this hypothesis. A limitation that authors noted from this study was that the specific CPT (CPT-3) used in this study only required a response to a single stimulus and therefore may not have been a task difficult enough to observe a difference between exposure groups.

In 6-year-olds, both studies found significant increases in commission errors, indicating increased impulsivity, while results for sustained attention were mixed. Both studies were well conducted in the two longitudinal cohorts. The approach to statistical analyses differed between the studies with Fried et al. using discriminant function analyses and Leech et al. using hierarchical modeling. As described by Fried et al. (1992a), their results suggest that prenatal exposure to cigarettes and cannabis may be associated differently with subsystems within the attentional process.

9-13 years

Fried et al. (1998) measured cognitive functioning in 9-12 year-old prenatally exposed children and used CPTs among a battery of other tests. Gordon Delay Tasks and Gordon Vigilance Tasks were used to measure impulsivity and sustained attention, respectively. The analysis found a significant association between children prenatally exposed to cannabis and scores related to the Gordon Delay Tasks, indicating increased impulsivity. No statistically significant association was observed for sustained attention.

Richardson et al. (2002) looked at five cognitive domains, one of which included attention and general mental efficiency via the CPT in children aged 10-13. Analysis showed that second trimester cannabis exposure significantly predicted more commission errors (increased impulsivity). Current maternal cannabis use was considered as a covariate and did not meet the criteria to be included in the final model for analysis. This study did not find statistically significant differences in omission errors indicating no differences between exposure groups in sustained attention.

Adolescence (13-18 yrs)

13-16 years

One study (Fried and Watkinson 2001) assessed the association between prenatal cannabis use and attention in 13-16-year old adolescents. Notably, this study did not find an association between prenatal cannabis exposure and impulsivity. This study conducted a wide range of tests and found significantly poorer stability in attention, which

measures consistency of attentional effort over time, in children prenatally exposed to cannabis. The study did not seem to control for postnatal cannabis use.

When looking across age groups, all studies using a CPT to assess impulsivity found an association between prenatal cannabis exposure and significantly increased impulsivity. Results regarding sustained attention are mixed or non-significant within all age groups.

Summary of studies using CPT:

Overall, four out of six studies found statistically significant associations between prenatal cannabis exposure and impulsivity in offspring. Three of these studies found associations specifically between prenatal cannabis exposure and increased errors of commissions on a continuous performance task (CPT). One study found an association between prenatal cannabis exposure and a higher score on the Conner's Impulsive-Hyperactive scale of Conner's Parent Rating Scale-48. Two studies that conducted CPT tasks found no association between prenatal cannabis exposure and impulsivity and none of the studies found an inverse relationship between exposure and impulsivity.

With regards to increased problems with sustained attention, two of six studies found a statistically significant association with prenatal cannabis exposure. One study measured sustained attention via omission errors (Fried et al, 1992a) and another combined several different test results into a composite score and found poorer "stability in attention" (Fried and Watkinson 2001). Another study found a significant positive correlation between prenatal cannabis exposure and errors in omission that was reduced to a marginally significant association with the addition of covariates into the model (Noland et al. 2005). Two studies (Fried et al 1998, Richardson et al. 2002) found no association between prenatal cannabis exposure and sustained attention, and another study (Leech et al., 1999) found improved sustained attention in children exposed prenatally.

The studies all use primarily CPTs to assess attention allowing for comparison between studies. The OPPS studies dichotomized cannabis use for analysis, while the MHPCD studies used trimester specific data and found that exposure during the 2nd trimester yielded statistically significant results. Noland et al. (2005) created an average severity score that combined levels of use across trimesters. The level of cannabis use in women during pregnancy may have differed between studies; however, it was not possible to compare the difference in exposure levels given the information provided in the publications.

These studies also come with some limitations that should be considered in determining the strength of evidence. Out of the four studies that found significant associations with prenatal cannabis exposure and impulsivity, one study did not control for postnatal cannabis use (Fried et al. 1992a). This is a significant limitation as other studies such as Noland et al. (2005) found that statistical significance diminished with the addition of

postnatal cannabis use of a caregiver. It is unclear in one of the OPPS studies whether the analyses included adjustment for postnatal cannabis exposure. The one study (Leech et al. 1999) that found improved sustained attention discussed limitations including lack of collection of reaction times in order to assess whether slower responses could account for the higher accuracy of responses.

The remaining five studies used varying methods to assess attention problems and yielded mixed results. The methods used in these studies included the Child Behavior Checklist for toddlers, Stroop tests, the Word Test, the Knox Cube Test, the Swanson, Noland, and Pelham (SNAP) questionnaire and the Behavior Rating Inventory of Executive Function-Teacher Form (BRIEF-TF). The CBCL and SNAP questionnaires were completed by mothers while the BRIEF-TF was completed by high school teachers. One out of the 5 studies measured child performance on tasks (Stroop Interference, the Word test, Knox Cube test).

Early Childhood (1-5 yrs)

One study (El Marroun et al. 2011) studied behavior and emotional problems in 18-month-old offspring. The Child Behavior Checklist for toddlers, which was used to assess behavior, contains a subscale dedicated to attention problems. The authors found a statistically significant increase in attention problems in prenatally exposed children. When stratified by gender, the relationship between prenatal cannabis exposure loses significance in boys but remains significant for girls. The CBCL questionnaire was filled out by mothers, which may not be an objective measure of the child's behavior.

Childhood (6-12 yrs)

The earliest study, O'Connell and Fried (1991), evaluated neurobehavioral development in 6-9 year old offspring with prenatal cannabis exposure. They conducted a battery of tests including the Stroop Color test, the Word Test, and the Knox Cube Test to assess attention. The discriminant function analysis identified Stroop interference as a factor discriminating between exposed offspring and unexposed offspring. However, when adjusted for covariates, including mother's personality and home environment, this association was no longer statistical significant.

Two studies were conducted within the MHPCD cohort that assessed attention problems in 10 year old offspring using SNAP questionnaires. Goldschmidt et al. (2000) evaluated the association between child behavior problems at 10 years of age, having mothers fill out the Swanson, Nolan, and Pelham (SNAP) questionnaire. The study found statistically significant associations for all of the following: 3rd trimester cannabis use was associated with higher scores in hyperactivity, attention, and impulsivity; 1st trimester cannabis use was associated with the attention scale of SNAP; and 1st trimester cannabis exposure was directly related to inattention symptoms in offspring and was indirectly related to delinquency. However, this study also did not control for the mother's behavioral

problems. Additionally, the SNAP questionnaire was filled out by mothers, which may not be an objective measure of the child's behavior.

The second MHPCD study, Goldschmidt et al. (2012), assessed the relationship between prenatal cannabis exposure and school achievement at 14 years of age. As a part of path analysis, attention problems at 10 years of age had previously been measured using the SNAP questionnaires. The study found that first trimester cannabis exposure increased attention problems at 10 years which was then significantly associated with lower school achievement at 14 years.

In this age group, 2 out of 3 studies found direct associations between prenatal cannabis use and attention problems in offspring. Both of these studies also linked the attention problems in offspring with larger behavioral problems.

Adolescence (13-18 yrs)

Rose-Jacobs et al. (2017) evaluated the effect of prenatal cannabis exposure on executive function in high school in the Boston City Hospital cohort. The test measure used was a Behavior Rating Inventory of Executive Functioning-Teacher Form completed annually by the student's teacher. The scale contained two subscales, the one pertaining to attention is the behavioral regulation subscale. This section assesses abilities to shift attention between different tasks, efficiently adapt to changing situations, and use inhibitory control to modulate strong or automatic behavioral/emotional responses. The study found that heavier prenatal cannabis exposure actually predicted better behavior regulation scores and metacognition scores. The authors state that explanations for these results "remain uncertain". However, this study also contained some limitations. The BRIEF-TF was filled out by the student's Language Arts teachers, which may not be an objective measure of behavior and ability. The number of women using cannabis was relatively small. Additionally, the focus of the study was to evaluate the association between intrauterine tobacco exposure and executive functioning.

Smith et al. (2004) evaluated response inhibition in 18-22 year old participants from the OPPS cohort by conducting an fMRI as participants engaged in a "Go/No-Go" task. This task requires participants to make a motor response to a particular stimulus (press x) and withhold response responding to a different stimulus. The study found no differences in omission or reaction time between exposed and unexposed groups. However, the cannabis exposed group had significantly more errors of commission than the non-exposed. Furthermore, imaging during the task showed a significant negative association between the amount of prenatal cannabis exposure and the neural activity during response inhibition in the anterior lobe of the left cerebellum. The study also observed increases in prefrontal cortex activity. The authors postulated that since prefrontal cortex activity increases during difficult tasks, this increase might be a compensatory response in exposed participants. The positive association observed (increased neural activity during the task) with the right premotor cortex is consistent with the known function of

this brain region (response inhibition, response competition, and the preparatory process leading to correct initiation or suppression of movement). The study also attempted to account for current cannabis use in adolescents and analyzed the 18 participants that did not test positive for cannabis in their urine samples. Similar trends were observed in this sample, although statistical significance could not be established due to small sample size.

These studies had mixed results. The three studies that used questionnaires that were completed by parents found significant results for attention. El Marroun et al. (2011) used CBCL and interestingly found significantly more attention problems in girls only. Both Goldschmidt et al. (2000) and Goldschmidt et al. (2012) found increased attention problems related to delinquency and lower school achievement, respectively. The one task-based fMRI study found a significant negative association between prenatal cannabis exposure and response inhibition (Smith et al. 2004). The one study that assessed performance on tasks found non-significant results after controlling for confounders (O'Connell and Fried 1991). Lastly, Rose-Jacobs et al. (2017) used teacher ratings and found better behavioral regulation in students.

Table D.15. Summary of Human Studies of Exposure to Cannabis and Δ9-THC and Developmental Outcomes: Attention

Cohort/ Study	*Sample size (n= exposed)*	*Sample characteristics*	*Age of offspring (yrs)*	*Exposure quantify-cation*	*Test used*	*Other assessment results*	*Impulsivity (commission error)*	*Sustained attention (omission errors)*	*Covariates*
OPPS Ottawa, Canada									
O'Connell and Fried 1991	n=28 regular Cnb users	Predominantly White, middle-class	6-9 yo	>1 joint/wk during pregnancy Range of exposure: ½ joint/wk- 50 joints/wk	Test Battery: TVPS Draw-A-Man Trail Making Test ***Stroop Color Word Test*** ***Knox Cube Test*** Motoric task WRAT Test of Language Development Passage Comprehen-sion from Woodcock Reading Mastery Test	Stroop Interference test was identified a discriminating variable in exposed vs unexposed differences, but was non-significant after adjustment (p=0.14)	N/A	N/A	Prenatal co-exposure (+) Postnatal Cnb exposure (-)
Fried et al. 1992a	n=126	Low risk, majority White, middle class	6	Dichotomized for analysis across entire preg	CPT: Gordon Vigilance Task Conner's Impulsive-Hyperactive scale of CPRS-48		Positive association with parental rating of impulsivity **F=2.1, p<0.05 one-tailed**	Positive association with total correct on CPT, and number of omissions on CPT in a dose-response manner **F=2.1, p<0.05 one-tailed**	Prenatal co exposure (+) Postnatal Cnb use (-)
Fried et al. 1998	n=131 90% Power Effect size: 0.30	Low risk, majority White, middle class	9-12	Dichotomized for analysis across entire preg	CPT: Gordon Delay and Gordon Vigilance Tasks		Positive association with Gordon delay efficiency ratio and Gordon delay total number of responses **F=7, p<0.01**	No significant association	Prenatal co exposure (+) Postnatal Cnb use at time of testing (+)

Cohort/ Study	*Sample size (n= exposed)*	*Sample characteristics*	*Age of offspring (yrs)*	*Exposure quantify-cation*	*Test used*	*Other assessment results*	*Impulsivity (commission error)*	*Sustained attention (omission errors)*	*Covariates*
Fried and Watkinson 2001	n=140 Effect size: 0.30 90% Power	Low risk, majority White, middle class	13-16	Dichotomized for analysis across entire preg	Varied Including: Conners (4) CPT Knox Cube Stroop Test Wisconsin Card Sorting Test Sentence Memory Test Seashore Test		No significant association	**F=5.2, p<0.01**	Prenatal co exposure (+) Postnatal Cnb use (unclear)
Smith et al. 2004	n=16	Low risk, majority White, middle class	18-22	Analyzed as a continuous variable as joints/wk Range of exposure: .33-54 joints/wk	fMRI as participants engaged in "Go/No-Go" task	Cnb exposed had significantly more commission errors: **F=6.24, p<0.02** Negative assoc between Cnb exposure and neural activity during response inhibition in anterior lobe of left cerebellum Separate analysis conducted on participants with no current Cnb use, same trends were observed	N/A	N/A	Prenatal co exposure (+) Postnatal Cnb use (+)
MHPCD Pittsburgh, PA									
Leech et al. 1999	N total 608	Half White and half African American, low SES	6	Dichotomized per trimester	CPT-3		2nd trimester **β=1.21, p<0.01**	2nd trimester **β= -0.56, p<0.05**	Prenatal co exposure (+) Postnatal Cnb use at time of testing (+)
Goldschmidt et al. 2000	1st trimester n = 235 2nd trimester n = 130	Half White and half African American, low SES	10-13	Dichotomized per trimester (none to light vs. moderate to heavy)	SNAP	3rd trimester exposure predicted lower scores on SNAP subscales: attention **(p<0.001**), hyperactivity	N/A	N/A	Prenatal co exposure (+) Postnatal Cnb use at time of testing (+)

Cohort/ Study	*Sample size (n= exposed)*	*Sample characteristics*	*Age of offspring (yrs)*	*Exposure quantify-cation*	*Test used*	*Other assessment results*	*Impulsivity (commission error)*	*Sustained attention (omission errors)*	*Covariates*
	3rd trimester n = 118					**(p<0.01),** impulsivity **(p<0.01)** 1st trimester exposure was associated with lower scores on SNAP subscales: **(p<0.01)** 1st trimester associated with increased inattention and delinquency			
Richardson et al. 2002	N total 593	Half White and half African American, low SES	10-13	Dichotomized per trimester	CPT-2		2nd trimester **β = 1.86, p < 0.05**	No significant association	Prenatal co exposure (+) Postnatal Cnb use (+)
Goldschmidt et al. 2012	N total 524	Half White and half African American, low SES	SNAP assessed at 10, School achievement at 14 yrs	Dichotomized per trimester	SNAP Score at age 10 WIAT School achievement	1st trimester →lower SNAP score (**standardized coefficient = -0.08, p<0.05**) → lower WIAT (**standardized coefficient = -0.11, p<0.005**)	N/A	N/A	Prenatal co exposure (+) Postnatal Cnb use (+)
Other									
Noland et al. 2005	85 (All 85 participants were co-exposed to cocaine)	Majority African American and low SES	4	Severity scores created based on frequency and pattern of use per trimester and across trimesters	CPT for preschoolers, modified		No significant association	Positive non-significant correlation β= 0.29, p=0.06	Prenatal co exposure (+) Postnatal Cnb use (+)
Rose-Jacobs et al. 2017	N total 131 n = 19 light exposure	89% African American and Caribbean, low-income, urban high school	High school students	Unexposed Lighter Heavier	Behavior Rating Inventory of Executive Functioning-	Heavier exposure predicted worse Behavior regulation			Prenatal co exposure (+) Postnatal Mother's

Cohort/ Study	*Sample size (n= exposed)*	*Sample characteristics*	*Age of offspring (yrs)*	*Exposure quantify-cation*	*Test used*	*Other assessment results*	*Impulsivity (commission error)*	*Sustained attention (omission errors)*	*Covariates*
	n = 15 heavy exposure				Teacher Form (BRIEF-TF)	scores: **β=-11.6, p= 0.01** Heavier exposure predicted worse metacognition scores: **OR=0.3, p = 0.04**			Substance use (unclear) Adolescent substance use (+)
El Marroun et al. 2011	N total 4077 n = 88 exposed (co-exposed with tobacco)	Large generation population cohort recruited in Rotterdam	1 1/2	Cnb exposure with tobacco co-exposure Tobacco only exposure in early preg Tobacco only exposure throughout preg No use of Cnb or tobacco during preg	CBCL	Increased attention problems: β=2.75, p=0.01 Increased attention problems in exposed girls: **β= 2.02, p=0.02**	N/A	N/A	Prenatal co exposure (+) Postnatal exposure (unclear)

Visual Function and Processing

Six studies, four of which were conducted in the OPPS cohort, examined aspects of visual perceptual functioning. Study details are provided in Appendix Table A.3. The first OPPS study, O'Connell and Fried (1991), assessed neurobehavioral development in 56 6- to 9-year-old children using the Test of Visual Perceptual Skills (TVPS) and other tests (O'Connell and Fried 1991). In discriminant function analysis, several visual variables stepped into the analysis: visual discrimination and visual sequential memory from the TVPS, and the Trail Making Test (which tests visual, conceptual, and visuomotor tracking). However, associations between prenatal cannabis use and poorer performance on these variables were not statistically significant after adjusting for confounding factors. Age of mother interacted with cannabis exposure such that children of younger mothers performed more poorly on the visual sequential memory test. Exposure was defined as use of at least one joint/week during pregnancy. There were 28 exposed mothers, who smoked between 1.5 and 50 joints/week during pregnancy, mean (SD)=14.4 (15).

The second OPPS study, Fried et al. (1998), evaluated cognitive function using the Wechsler Intelligence Scale–III (WISC-III) to assess 9- to 12-year-old children (Fried et al. 1998). They reported based on a discriminant function analysis that lower WISC-III Block Design and Picture Completion scores distinguish children of prenatal cannabis users from children of non-users, although Picture Completion mean scores were not significantly different for exposed and unexposed children. Fried et al. state that the Block Design subtest requires perceptual organization, spatial visualization, and abstract conceptualization, and the Picture Completion subtest requires the ability to differentiate essential from nonessential details. Combined, these subtests may involve both basic visuospatial and visuo-motor abilities along with higher order cognitive processes including planning, impulse control, visuo-construction and visuo-analysis. To explore whether *basic* visual motor and spatial functioning was affected, the authors administered the Developmental Test of Visual-Motor Integration. The results suggested that prenatal cannabis exposure is not associated with the basic aspects of visual functioning, and the authors interpreted the persistent association of prenatal cannabis exposure with the Block Design and Picture Completion subtests as evidence of an adverse effect on higher order cognitive processes.

The third OPPS study, Fried and Watkinson (2000), examined visuoperceptual skills with another battery of tests in (presumably) the same 9- to 12-year-old children as in Fried et al. (1998) and Fried and Watkinson (2000). Prenatal cannabis exposure was not associated with any of the TVPS subtests or with the overall perceptual quotient, suggesting no differences in basic, fixed, functional visuoperceptual abilities. However, children prenatally exposed to cannabis performed significantly more poorly on WISC-III

Block Design and Object Assembly, which the authors state require skills in planning, integration, analysis, and synthesis, in addition to basic visuoperceptual abilities. Fried and Watkinson interpret these findings to suggest that prenatal cannabis exposure is associated with poorer performance in "'top-down' integrative visuoperceptual processing—the type of neurocognitive requirement underlying executive function".

A fourth OPPS study, by Smith et al. (2006), investigated associations between prenatal cannabis exposure and visuospatial working memory in 31 18- to 22-year-olds. Prenatally exposed and unexposed participants performed similarly on Visuospatial n-back and Match to Centre tests. However, the fMRI imaging results found significantly more neural activity in the left inferior and middle frontal gyri, left parahippocampal gyrus, left middle occipital gyrus and cerebellum, and decreased neural activity in the right inferior and middle frontal gyri in prenatally exposed participants. The most robust effect was lower activity in the right precentral gyrus/premotor cortex with increasing prenatal cannabis exposure. This brain region reflects the encoding process in visuospatial short term memory. Smith et al. interpret the data as suggesting that prenatal cannabis exposure alters neural functioning during visuospatial working memory processes in young adulthood, and that differences in brain activity among prenatally exposed participants may be due to strategic differences in the approach to performing the task, or a compensatory response where left brain systems are increasingly engaged to compensate for functionally compromised right brain systems.

Willford et al. (2010b) evaluated the association between prenatal cannabis exposure and processing speed, visual-motor coordination, and interhemispheric transfer in 16-year-old participants in the MHPCD study (Willford et al. 2010). First trimester exposure to cannabis was associated with slower processing speed on the bimanual coordination task, which measures the efficiency of interhemispheric information transfer. Third trimester exposure was associated with poorer interhemispheric motor coordination, indicated by slower reaction times on one measurement. Third trimester cannabis exposure was also associated with faster reaction times on a measure of symmetrical movement, indicating better visual motor coordination.

Chakraborty et al. (2015) studied the association between prenatal cannabis exposure and global motion perception thresholds in children around 4.5 years old (Chakraborty et al. 2015). Children prenatally exposed to cannabis were observed to have better motion coherence thresholds than unexposed children. Global motion perception thresholds are related to visuomotor tasks such as reaching and grasping. Exposure to cannabis was reported to lessen or cancel out the negative effect of alcohol exposure. The authors discussed the possibility that prenatal cannabis exposure might improve dorsal stream function (involved in information processing) but impair ventral stream function. Chakraborty et al. (2015) also propose an alternative explanation – that cannabis may

not impair visual processing at the dorsal level but rather may affect other processes such as visual attention, memory, or response inhibition rather than visual perception.

Some limitations of the studies include lack of control for postnatal environmental cannabis exposure (Fried and Watkinson 2000, Chakraborty et al. 2015, O'Connell and Fried 1991), which could be a possible confounder of results but might be difficult to implement with smaller samples. Willford et al (2010b) also did not control for environmental cannabis smoke in their sample of 16-year-olds, but did control for the participants' use of cannabis. Some studies had small samples, one of which reported non-statistically significant trends associated with cannabis exposure after adjustment for confounders (O'Connell and Fried 1991).

Fried and colleagues have proposed that while basic visual functions are relatively unaffected by prenatal cannabis exposure, higher order cognitive processes related to visual analysis might be affected by this exposure. Three OPPS studies reported that basic visual functions were not significantly different between exposed and unexposed groups after accounting for covariates (O'Connell and Fried 1991, Fried et al. 1998, Fried and Watkinson 2000). Two of these studies reported that prenatal cannabis exposure appeared to negatively affect higher order cognitive processes related to visuoperceptual skills such as visual analysis and hypothesis testing (Fried et al. 1998, Fried and Watkinson 2000).

Willford et al. (2010b) reported some improvement in visual motor coordination involving symmetrical movement, as well as slower processing speeds and interhemispheric motor coordination with different trimesters of cannabis exposure. Finally, although Smith et al. (2006) found no difference in performances on assessments of prenatally exposed vs. unexposed young adults, there were differences in neural activity, indicating differences in neural functioning in young adulthood.

Intelligence / Achievement

A number of studies examined the effect of mother's exposure to cannabis during pregnancy on various measures of intelligence. The detailed summary tables of these studies can be found in Appendix Table 2.4.

Early childhood

The impact of prenatal cannabis exposure on intelligence in early childhood (ages 1-5) was investigated in three prospective cohort studies, each using varying assessment methods.

Fried and Watkinson (1988) reported maternal cannabis use during pregnancy was negatively associated with Reynell Comprehension scores in young children. No association was observed with the Mental Development Index.

Day et al. (1994b) used the Stanford-Binet Intelligence Scale (SBIS) as a method of assessing intelligence and cognitive ability in 3 year-old offspring whose mothers reported cannabis use during pregnancy. While the study did not find a significant association with the SBIS composite score for any trimester, significant associations were observed in analyses evaluating race and preschool/daycare attendance as interaction terms. Prenatal cannabis exposure was associated with decreased subscale scores in verbal reasoning and short-term memory in African American but not white offspring. The interaction term between average daily joints per day consumed by mothers during pregnancy and preschool or day care attendance of offspring was significant. This analysis found that the composite, short-term memory, and verbal reasoning subscales were reported significant in the interaction model and dependent on race and preschool attendance of the child. A significant negative association was observed between prenatal cannabis exposure and performance on the SBIS, which was offset by preschool/day care attendance, in white but not African-American children.

Fried and Watkinson (1990) assessed neurobehavior in 3-4-year old children using the McCarthy Scales of Children's Abilities and Reynell Development Language Scales in 3 year-olds, as well as the Peabody Vocabulary test in 4 year olds. Discriminant function analysis showed that the Peabody Vocabulary test and McCarthy memory and verbal subscales were statistically different discriminant functions. Lower mean scores on the Peabody Vocabulary Test were observed in the group reporting heavy cannabis exposure during pregnancy compared to low or moderate exposure groups. The lowest memory and verbal subscales scores from the McCarthy scales were found for moderate not heavy exposures. The sample sizes in both groups were small. No associations were found for abstract or visual reasoning scores.

Childhood

Eight prospective cohort publications report on the results of intelligence testing in children of mothers who used cannabis during pregnancy. All of the studies were of participants in either the OPPS or MHPCD cohorts.

Fried et al. (1992b) used the McCarthy Scale of Children's Abilities and Peabody Picture Vocabulary test to assess cognitive and receptive language development in 5-6 year old offspring. The study found no statistically significant association between prenatal cannabis exposure and cognitive function.

Goldschmidt et al. (2008) measured the intelligence of 6 year old offspring via SBIS. The analysis found lower composite scores, verbal reasoning scores, and quantitative reasoning scores and short term memory scores for children whose mothers used cannabis during pregnancy. The only subscale for which there was no association was the abstract/visual reasoning subscale. Prenatal cannabis exposure during the first trimester was associated with a deficit in verbal reasoning. Additionally, out of the subscales that yielded significant relationships, lower scores for three of the four

subscales were observed for second trimester exposure, as well as for the third trimester on the quantitative reasoning subscale.

O'Connell and Fried (1991) studied neurobehavioral development in 6-9 year old offspring including assessments of behavior problems, language comprehension, and visual-perceptual outcomes. The study used WISC-R as a global intellectual measure, and the syntax quotient from the test of language development. Academic achievement was assessed via the Wide Range Achievement Test-Revised (WRAT-R). No statistically significant findings were observed for prenatal cannabis exposure. Results of the discriminant function analysis found seven outcome variables that discriminated between the cannabis exposed offspring and unexposed offspring, explaining 35% of the variance. Measures that discriminated between children prenatally unexposed and those exposed to cannabis included language comprehension and distractibility. This study did not control for postnatal cannabis use.

Richardson et al. (2002) studied varying cognitive domains in offspring prenatally exposed to cannabis at age 10. The domains included problem solving and abstract reasoning, assessed by Wisconsin Card Sorting test, learning and memory assessed by WRAML, and SBIS to assess intellectual development. Regression analyses found that learning and memory scores were significantly lower in offspring prenatally exposed to cannabis in the first trimester, with specifically lower scores in design memory and screening index. This study found no associations between prenatal cannabis exposure and problem solving, abstract reasoning, mental flexibility, psychomotor speed and eye-hand coordination.

Goldschmidt et al. (2004) assessed academic achievement and underachievement in 10 year old offspring. Underachievement was defined as a large disparity between intelligence and school achievement. Achievement was assessed by WRAT-R and PIAT-R as well as teacher's report. The expected level of achievement was based on their intellectual ability as measured by SBIS. Regression analysis found that first trimester use predicted poor scores on the WRAT-R reading scores and spelling scores as well as lower teacher's rating. Using structural equation modeling, first trimester cannabis exposure significantly predicted child's psychological status which then significantly predicted achievement scores. However, there was no direct association between first trimester cannabis exposure and achievement. Second trimester exposure to cannabis was associated with lower PIAT-R reading comprehension scores, lower teacher evaluation of performance, and underachievement. However, this association was not found when structural equation modeling was used. Third trimester cannabis exposure was not associated with any measures of academic achievement. The authors noted that while cannabis was prevalent, the women in the study were not heavy users of cannabis.

Fried et al. (1997) assessed reading and language in 9-12 year-old offspring using WISC-III, WRAT-R, the Peabody picture vocabulary test, a passage comprehension assessment, oral cloze task, and regular and exception pseudoword task. Prenatal cannabis use was not associated with deficits in reading or language. Discriminant function analysis resulted in one significant function accounting for 83% of the variance between exposed groups and unexposed groups. The one significant discriminant function were variables from the Pseudoword task- specifically the total correct scores and the phonological scores. Offspring in the moderate exposure cannabis group were reported to perform worse than those in both the no exposure and heavy exposure groups. The analysis reported non-significant associations for the other reading and language variables.

Fried et al. (1998) studied cognitive and executive function as well as differential effects on global intelligence compared to executive function in 9-12 year old children of mothers exposed during pregnancy. The study used: WISC-III and Fluency Test; Category Test, which examines the ability to adjust behavior on the basis of negative and positive feedback; and Gordon Delay/Vigilance test. Significant results were reported for the category, Gordon delay, WISC-Block Design, and WISC-Picture completion tests, based on discriminant function analysis. These relationships persisted after controlling for potential confounders including postnatal cannabis exposure.

Fried and Watkinson (2000) primarily examined visuo-perceptual abilities of offspring, but includes the WISC-III score that assesses some indicators of intelligence. The study found significantly worse scores on the perceptual organization index of WISC-III, Block Design subscale, and Object Assembly subscale. These subcales require skill in planning, integration, analysis, and synthesis and the authors consider these findings consistent with the hypothesis that prenatal cannabis exposure impacts aspects of executive functioning.

Overall in this age group, intelligence was measured with a large variety of tasks and questionnaires and yielded mixed results. All but three of these studies controlled for postnatal cannabis exposure via mother's continued use (O'Connell and Fried 1991, Fried et al. 1992b, and Fried and Watkinson 2000).

Out of the eight studies, seven found statistically significant negative relationships with at least one of the measures assessed in the study. Some of the common assessments used included WISC-III, WRAT-R/PIAT-R, SBIS, and Peabody Vocabulary tasks. Of the tests that used WISC-III two studies found statistically significant results (Fried et al. 1998 and Fried and Watkinson 2000). Both studies found significantly lower WISC-Block Design Scores, and one study of the two found lower WISC- picture completion scores and lower WISC-Object Assembly scores. Out of the three studies that used WRAT-R as a measure of academic achievement (O'Connell and Fried 1991, Goldschmidt et al. 2004, Fried et al. 1997), one study found significantly lower WRAT-R reading, spelling,

and reading comprehension scores (Goldschmidt et al. 2004). Three studies used SBIS as a method of measuring intelligence in children (Goldschmidt et al. 2008, Richardson et al. 2002, and Goldschmidt et al. 2004). Of these studies, Goldschmidt et al. (2008) reports lower composite scores, verbal reasoning scores, quantitative reasoning scores, and short term memory scores. Goldschmidt et al. (2004) reported significant underachievement which they related to their previous findings of inattention symptoms (Goldschmidt et al., 2000). The Peabody Vocabulary Test was used in two studies, Fried et al. (1992b) and Fried et al. (1997), with neither yielding statistically significant results.

Adolescence

Three publications reported prospective cohort studies on intelligence scores of adolescents whose mothers used cannabis during pregnancy.

Fried et al. (2003) evaluated the association between prenatal cannabis exposure and cognitive functioning in 13-16 year old offspring. The study used WRAT and Peabody Spelling to assess general reading and language skills, as well as WISC-III to assess overall intelligence. They also used Missing Numbers, Abstract Designs, Sentence Memory, and Knox Cube tasks to assess different aspects of auditory and visual memory. The only outcomes that were statistically significant in the discriminant function analysis were Abstract designs and Peabody Spelling tasks.

Goldschmidt et al. (2012) assessed school achievement in 14 year old offspring. The study used the WIAT test to measure school achievement and assessed several potential mediators of school achievement including SBIS, CDI, SNAP, and Health Behavior Questionnaire as measures of cognitive development, depressive symptoms, attention problems, and substance use, respectively. The study found first trimester cannabis exposure was directly associated with a lower WIAT score. After running regression analyses with mediational models, the study found SNAP scores, early initiation of substance us, depressive symptoms, and SBIS scores to be significant mediators of the relationship between first trimester cannabis exposure and school achievement at age 14. First trimester cannabis use was found to significantly predict each of these mediators, and these mediators consequently predicted lower school achievement. Another regression was performed, and after dichotomizing cannabis exposure and controlling for significant covariates WIAT subscales yielded significantly lower composite scores and reading subscale. The study also assessed whether second or third trimester exposures would predict academic performance and did not find an association.

Rose-Jacobs et al. (2017) evaluated executive functioning in high-schoolers who had been prenatally exposed to cannabis as a part of a study that was mainly assessing tobacco. The study categorized executive function into two subscales, behavioral regulation and metacognition (behavioral regulation scores are discussed in the Attention topic summary). Metacognition scores indicate higher order thinking such as the ability to

understand, analyze and control one's cognitive processes in order to manage performance. The study observed better behavior and metacognition scores in cannabis exposed high school students.

Neuroimaging

As detailed in Appendix Table 2.5, six studies conducted neuroimaging; one study from the MHPCD cohort examined structural changes (Wilford et al, 2010b), one from the Generation R cohort examined brain morphology (El Marroun et al., 2016), a study from the Boston Hospital cohort (Rivkin et al., 2008) and three studies from the OPPS cohort used fMRI to examine brain activity (Smith et al. 2004, 2006, 2016). The three OPPS publications studied a subsample of 31 children from the cohort. Smith et al. (2016) incorporated the data from Smith et al. (2004) and Smith et al. (2006), and as such these earlier two publications will not be separately reviewed here. A strength of this dataset is that the studies were conducted in three separate longitudinal cohorts with populations differing in demographics, such as SES, education, ethnicity, and country of residence.

The primary focus of the study by Rivkin et al. (2008) was prenatal cocaine exposure and brain volume. The study included 10-12 year old children from the Boston City Hospital cohort (1990-93). The number of children prenatally exposed to cannabis, assessed through self-report or biological assay, was small (8 cocaine exposed children and 3 cocaine non-exposed children). Results of the study showed no statistically significant association between prenatal cannabis exposure and total brain volume, gray matter, white matter, subcortical gray matter, cerebrospinal fluid or parenchymal volume after adjustment for demographic factors and other substance use. An analysis of variance to examine the cumulative effect of exposure to more than one substance found that the smallest volumes of cerebral cortical gray matter, total parenchymal volume, and head circumference were associated with prenatal exposures to all four substances: cocaine, cigarettes, alcohol, and cannabis.

In the study by Willford et al. (2010b), structural changes in the caudate nucleus were studied in 45 young adults recruited from the MHPCD cohort. The focus of the study was prenatal alcohol exposure; prenatal cannabis exposure was included to determine whether it had a modifying effect (number exposed prenatally to cannabis was 20). No association was seen between prenatal cannabis exposure and caudate asymmetry, nor was there any interaction with prenatal alcohol exposure, which did have association with caudate asymmetry.

A study by El Marroun et al. (2016) was conducted in 6-8 year olds from the Generation R cohort in the Netherlands. Brain morphology, using MRI neuroimaging, was studied in children prenatally exposed to cannabis as well as in unexposed children. No significant differences were observed between the two groups for measures of total brain volume, gray matter volume, or white matter volume. However, children prenatally exposed to

cannabis were observed to have thicker cortices, specifically in the superior frontal area of the left hemisphere and a thicker frontal pole in the right hemisphere in comparison to control non-exposed children. The authors noted that their results were “in line” with the findings of Smith et al. (2004, 2006). The authors offered a possible interpretation of the finding of a thicker prefrontal cortex in the cannabis-exposed children as being the result of altered neurodevelopmental maturation. However, it was also noted that there was a high degree of co-occurrence of cannabis and tobacco exposure, making it difficult to attribute the observed effects in the cannabis exposed children to cannabis exposure only.

In the study by Smith et al. (2016), 18-22 year olds performed four executive functioning tasks while in the fMRI scanner. The small sample of young adults included 15 who had no prenatal cannabis exposure, and 16 who had been exposed prenatally. The results from a response inhibition task and a visuospatial working memory task were published previously (Smith et al., 2004, 2006). In the 2016 paper, data from these two tasks were reanalyzed “with more rigorous and up to date methods” and are presented together in this paper with results from the two additional fMRI tasks that had not been previously published. These included a letter 2-back working memory task and an interference Counting Stroop task. Performance on the tasks was not significantly different between the two groups. The results showed that for all four executive functioning tasks the prenatally exposed group had significantly more brain activity compared to the non-exposed group, specifically in the left posterior region of the brain. The authors stated that this suggests the “need for a compensatory response whereby either additional brain regions are required to perform the tasks or more activity in typically activated regions is necessary.”

E. Animal Studies of Developmental Toxicity

A number of *in vivo* studies in animals have been conducted to investigate the potential developmental toxicity of cannabis smoke and Δ^9-THC. Multiple studies have also been conducted using *ex vivo* and *in vitro* animal model systems. The endpoints evaluated in these studies include effects on embryo development and implantation, later stages of embryo and fetal development, neurodevelopment and behavior, effects in zebrafish embryos or larvae and epigenetic marks and gene expression.

Studies on Somatic Development

Early Embryo Development and Implantation

Early pregnancy involves molecular interactions among numerous systems (e.g., immune, EC), cell types, and signaling pathways as described in Section C (Overview of the EC System. Embryo development and implantation). Embryonic development and successful implantation requires coordination between components of the EC system in the oviduct and endometrium with those in the embryo.

CB_1R and CB_2R are expressed in early embryos (Paria et al. 1995), fetal membranes, the reproductive tract, and the placenta (Taylor et al., 2007). Studies of mouse embryos have detected CB_1R mRNA in preimplantation 4-cell embryos through the blastocyst stage, and CB_2R mRNA has been detected in mouse 1-cell embryos through the blastocyst stage (Paria et al. 1995). This suggests that the embryo may express CBRs from as early as gestational day (GD) 1. Indeed, CB_1R signaling has been shown to play a crucial role in oviductal transport of the embryo (Maccarrone 2008; Sun and Dey 2008).

Endogenous cannabinoids such as AEA are produced locally by the uterine tissue of mice (Paria et al. 1995; Paria et al. 1996; Schmid et al. 1997) and humans (Scotchie et al. 2015). The levels of uterine AEA fluctuate with changes in pregnancy status (Schmid et al. 1997), and low levels of AEA, as well as CB_1R expression have been shown to be essential for mouse embryo implantation (Paria et al. 2001; Wang et al. 1999).

Given that AEA and CB_1R interaction is needed for successful embryo implantation, and that Δ^9-THC has been demonstrated to interact with CB_1R receptors expressed by the embryo, it has been hypothesized that Δ^9-THC could interfere with successful embryo development and implantation. One *in vitro* study (Paria et al. 1995), and three *in vivo* studies (Paria et al. 1992; Paria et al. 1998; Paria et al. 2001); (Paria et al. 1995) from the same research group have evaluated the effects of Δ^9-THC exposure during embryonic development and implantation in a mouse model. The design and an overview of the results of these studies are presented in Appendix Table 3.1.Error! Reference source not found.

As shown in Appendix Table 3.1, between 60% to 89% of 2-cell mouse embryos did not develop to blastocyst stage after *in vitro* exposure to Δ^9-THC (Paria et al. 1995). However, no effect on implantation was observed in mouse after THC exposure in vivo (Paria et al. 1992). Yet when in a subsequent experiment the animals were exposed to P450 enzymes inhibitors (to prevent THC metabolism by P450 enzymes) only one out of 13 mice had implanted blastocyst (Paria et al. 1998). Under the same experimental conditions, the co-exposure with a CB_1R inhibitor recovered the implantation rate completely (Paria et al. 1998). Finally, to continue testing the hypothesis that the THC effect is mediated by CBRs, a CBR mutant mouse model was used. Under these experimental conditions, none of the wild type mice (0/5) had implantation sites while the CBR mutant had an 83% (5/6) of embryos implanted (Paria et al. 2001).

General Effects from Whole Animal Developmental Toxicity Studies

Thirty-eight whole-animal developmental toxicity studies investigating multiple potential effects of prenatal exposure to cannabis smoke or Δ^9-THC on general development were identified and retrieved. These apical-type studies were published between 1971 and 2017. The majority were conducted during the 1970s, with only two published after 2000.

To streamline presentation of the data from these whole-animal developmental toxicity studies, their methods and results have been summarized in tabular form and are presented in several Appendix Tables within Appendix 3, various periods (embryo in vitro, pre-natal, postnatal) and routes of exposure (inhalation, oral, injection).

A brief overview of the findings from these studies is provided below.

Cannabis Smoke

Five published studies, some reporting on several experiments, exposed pregnant rodents to cannabis smoke (See Appendix Table 3.2). Only the newest study (Benevenuto et al. 2017) employed a “nose-only” inhalation exposure apparatus, the others used full body chamber exposures. The full-body method does not prevent animals from receiving oral, as well as inhalation exposure as they may lick test-substance residue off their fur.

Seven out of nine experiments reported at least one statistically significant adverse outcome on offspring following prenatal cannabis smoke exposure. These outcomes included (number of experiments in which specified outcome was reported):

- Altered sex ratio (1).
- Increased resorptions or stillbirths (2).
- Delayed postnatal developmental landmarks (4).
- Decreased birth weights, and decreased pup weight gain postnatally (4).

Taken together, results from these studies are consistent with an effect of prenatal exposure to cannabis smoke on both pre- and postnatal growth. Delays in acquisition of postnatal developmental landmarks also suggest an association between exposure and generalized growth retardation.

Confidence in the data is hampered by inadequate reporting and poor experimental methodology. All the studies noted above included one or more of the following specific deficits:

- Inadequate or marginally adequate group size.
- Analysis on a total group basis, rather than per litter.
- Lumping all dose groups together for comparison to controls.

Only one study of gestational exposure of rodents to cannabis smoke employed treatment groups larger than 10 pregnant animals. Rosenkrantz (1999) started with 30-50 mice per group, but combined data from groups exposed to cannabis smoke at doses calculated to deliver 0.8, 2.6, and 3.8 mg Δ^9-THC/kg-bw for statistical comparison to controls (Rosenkrantz 1999). Resorption and mortality data for the total combined group of exposed offspring were compared to controls, without regard to dose or litter.

Sample size is one of the key factors in determining the power of a study to detect a true effect. The U.S. EPA Risk Assessment Guidelines (1991) cite the work of Nelson and Holson (1978) to support a recommendation that appropriate test protocols for animal developmental toxicity studies specify approximately 20 pregnant females per each control and exposed group at the time of evaluation (Nelson and Holson 1978; US EPA 1991). These numbers are anticipated to provide minimal detectable changes of an increased *in utero* death rate per litter of three to six times above controls, or decreased mean fetal weights per litter of 0.15 to 0.25 times control weights. Use of fewer animals per group results in a consequent loss of power.

The litter, rather than an individual pup, is considered the appropriate statistical unit for developmental toxicity studies (US EPA 1991). This is because the dam is the exposed individual, and the litter is a strong determinant for offspring outcomes such as viability/mortality, fetal/birth weight, and malformation frequency. Particularly combined with small sample size, failure to account for litter effects can allow a small proportion of outlier litters to give a skewed impression of a dose group.

All of the studies noted above as reporting significant adverse effects of exposure to cannabis smoke performed their analyses on a per group, not per litter basis. Where analysis was performed on a per litter basis, statistical significance was not achieved.

Oral Δ^9-THC

Eighteen published studies, some reporting on multiple experiments, exposed rodents or rabbits to Δ^9-THC by the oral route of exposure. (See Appendix Table 3.3). One study

did not clearly specify the method of oral exposure, while the rest specified gavage. A nineteenth study was conducted in chimpanzees by an unspecified method of oral exposure.

Twenty out of 27 rodent or rabbit experiments reported at least one statistically significant adverse outcome on offspring following gestational oral exposure to Δ^9-THC. These outcomes included (number of experiments in which specified outcome was reported):

- Altered sex ratio (1).
- Increased fetal, perinatal, or postnatal offspring mortality (8).
- Reduced postnatal weight gain (1).
- Decreased fetal or birth weights (7).
- Increased external malformations (2).
- Altered hormone levels or fertility in F1 males following prenatal exposure (6).

Fleischman et al. (1980) reported on three experiments using a rat model, and a fourth experiment using mice. One of the rat experiments involved 50 pregnant animals per dose group and controls, with oral exposure to 0, 12.5, 25, or 50 mg Δ^9-THC/kg-day on GD 6-15 (Fleischman et al. 1980). The mouse experiment (N = 90-95/group) used higher doses of 0, 150, 300 or 600 mg Δ^9-THC/kg-day on GD 6-15. Dams of both species were sacrificed at different times during gestation: GD 8, 11, 14, 17, or 19.

Mean numbers of implantation sites and viable fetuses were presented on a per litter basis. The overall pattern of results was similar for both species: while implantation frequency per litter did not appear to be influenced by dose of Δ^9-THC, the numbers of viable fetuses per litter appeared to decrease with increasing dose. For mice, the decrease in fetal viability was greater than that seen at the lower doses used in rats, with no mouse fetuses surviving gestational exposure to the high dose of 600 mg/kg-day. It should also be noted that data for animals sacrificed on different gestational days were lumped by dose group such that animals in a group were exposed to the same daily dose of Δ^9-THC, but not necessarily the same total gestational dose.

Several studies from one research group (Dalterio et al. 1984; Dalterio et al. 1986a; Dalterio and deRooij 1986; Dalterio et al. 1986b; Dalterio et al. 1999) looked specifically at effects of premating (F0 males only) or gestational (F0 females only) oral Δ^9-THC exposure on male reproductive outcomes of adult F1 offspring. Reported findings of note include:

- Reduced fertility.
- Decreased testosterone.
- Increased liver weights.
- Increased liver cytochrome P-450 concentration.

Confidence in the findings is undermined by poor reporting, including repeated failure to specify the number of animals in each original treatment group and/or to clearly account for either litter-origin or numbers of offspring included in the final analysis.

An additional study from this group, Dalterio and Bartke (1981), reported significant increases in fetal deaths as well as decreased testosterone in male fetuses exposed to Δ^9-THC on GD 12-16 and evaluated on GD 16 (Dalterio and Bartke 1981). Confidence in the findings is limited by failure to report the number of maternal animals included in the treated and control groups.

The chimpanzee study (Grilly et al. 1974) reported pregnancy outcomes following pre-mating Δ^9-THC-exposure of males and/or females. All three mating combinations (treated male and female; untreated male and treated female; treated male and untreated female) resulted in at least one live-born offspring. Due to limited reporting of methods and results, meaningful comparisons to colony norms for untreated animals cannot be made.

Injected Δ^9-THC

Thirteen published studies, some reporting on multiple experiments, exposed rodents or rabbits to Δ^9-THC by an injection route of exposure. A fourteenth study was conducted by injection in pregnant rhesus monkeys. (See Appendix Table 3.5)

Fourteen out of 16 experiments reported at least one statistically significant adverse outcome on offspring following prenatal exposure to injected Δ^9-THC. These outcomes included (number of experiments in which specified outcome was reported):

- Altered sex ratio (1).
- Increased fetal, perinatal, or postnatal offspring mortality (8).
- Decreased fetal or birth weights (9).
- Decreased postnatal growth (1).
- Increased external malformations (1).
- Delayed appearance of developmental landmarks (1).

Overall confidence the data set is constrained by limitations in experimental study design and reporting. Most used test groups of marginal size and failed to perform statistical analysis on a per litter basis.

The single study in rhesus monkeys (Asch and Smith 1986), used five sexually mature females per group, and dosed with Δ^9-THC by intramuscular injection (i.m.) starting on the day of confirmation of pregnancy (around GD 21-35) and continuing throughout gestation. Four out of five pregnancies were lost in treated animals: three by early spontaneous abortion, and the fourth was stillborn. Vehicle controls produced five live-

born infants out of five pregnancies. An additional experiment where pregnant animals were exposed to Δ^9-THC later in pregnancy resulted in five out of five livebirths to pregnant treated females, though one infant died at two days postnatal age.

The authors concluded that the early pregnancy loss with Δ^9-THC might have been related to "a premature decline in [chorionic gonadotropin] mCG and progesterone concentrations…". Information on the reproductive histories of the individual females used in the studies was not provided, and might have been useful for interpretation. Colony statistics for pregnancy loss versus live births would also have been helpful.

Immune System Effects

Evidence for effects of gestational cannabinoid exposure on the immune system in offspring have recently been reviewed (Dong et al. 2019; Zumbrun et al. 2015). Most of the evidence is indirect and will be briefly discussed with reference to recent reviews in section B.2.2.2.1. below. The single study providing direct evidence of effects on the developing immune system from prenatal exposure to Δ^9-THC (Lombard et al. 2011) will be discussed in some detail in the later in the section.

Indirect Evidence on Δ^9-THC Immune System Effects

Adult and fetal immune cells express both CB_1 and CB_2 receptors (as reviewed by (Dong et al. 2019)). Interactions of these receptors with exogenous cannabinoids can alter cytokine levels, leading to apoptosis of lymphoid cells and induction of immune suppressor cells. Gestational exposures to exogenous cannabinoids may lead to dysregulation of immune function, having consequences for defenses against infections in postnatal offspring (Lombard et al. 2011).

One experimental study del Arco et al. (2000) as reviewed by (Dong et al. 2019)), exposed pregnant and lactating rats to the synthetic cannabinoid HU-210, which is an agonist for CB1 and CB2. Treatment altered development of the immune system and led to long-lasting effects on the function of the hypothalamus-pituitary-adrenal axis. Prenatally exposed adult male offspring showed a reduced T-helper subpopulation in the spleen, and a dose-related decreased ratio of T-helper/cytotoxic T cells in peripheral blood.

Direct Evidence on Δ^9-THC Immune System Effects

Lombard et al. (2011) used pregnant C57BL/6 mice to study the effects of gestational exposure to Δ^9-THC on the development of offspring thymiccellularity and function (Lombard et al. 2011). Specific effects on the developing immune system were teased out in a series of focused experiments documenting the following:

- Mouse fetal thymocytes express high levels of CB1 and CB2 receptors.

- Caspase-dependent apoptosis caused thymic atrophy and altered T cell subpopulations following Δ^9-THC exposure on GD 16.
- *In vivo* receptor blocking experiments showed that pretreatment with antagonists attenuated Δ^9-THC-induced immunological changes.
- Significant immune dysregulation was demonstrated by decreased proliferative and antibody responses to human immunodeficiency virus (HIV) gp120 antigens.

Seven specific experimental protocols and results from the Lombard et al. (2011) paper are described below:

A. Messenger RNA (mRNA) from thymocytes of GD16 fetuses was compared to adult thymocyte mRNA using reverse transcription polymerase chain reaction (RT-PCR). Adult and fetal cells showed similar patterns of CB_1R and CB_2R mRNA expression. CB_2R message was expressed at much higher levels than CB_1R in both fetal and adult samples.

Selection of GD 16 as the focus of treatment and early assessment was explained as corresponding to the initial stages of fetal T cell development. Therefore, GD 16 was presumed to be a sensitive window for toxicants to disrupt development of the immune system.

B. Thymic cellularity was evaluated following intraperitoneal (i.p.) injection of 20 or 50 mg/kg Δ^9-THC on GD 16. GD 17 fetal thymi showed a dose-dependent decrease in total cellularity (see **Figure 5** below). The effect was considered indicative of thymic atrophy.

Figure 5. Thymic Cellularity in GD17 Mouse Fetuses Following Δ9-THC treatment on GD16.

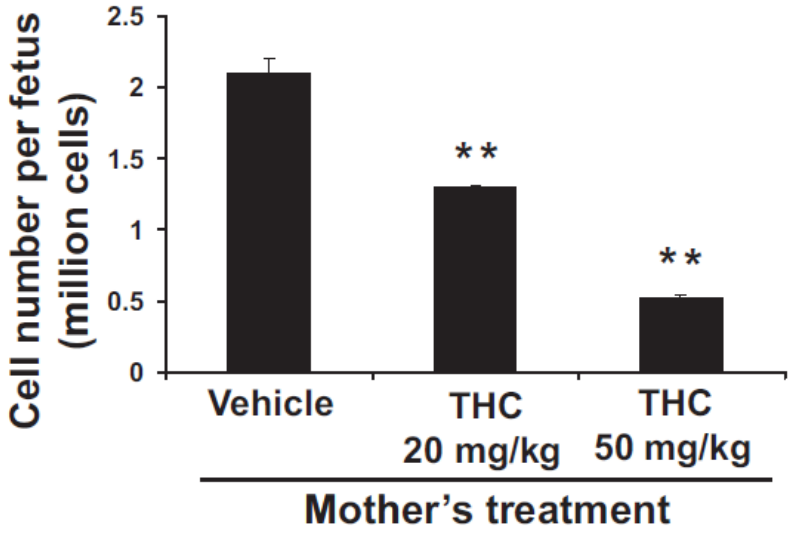


Figure 5. From Lombard et al. (2011). On GD16, groups of two C57BL/6 pregnant mice were given Δ^9-THC (20 or 50 mg/kg) or vehicle by i.p. injection. Control dams were given injections of the dimethyl sulfoxide (DMSO) vehicle. On GD17, thymi of fetuses from each pregnant mouse (average 10) were harvested and pooled separately for analysis. Viability of thymic cells was determined by trypan blue dye exclusion. Bars represent the mean thymic cellularity per fetus S.E.M., $p < 0.0062$, one-way ANOVA. **, statistically significant difference from vehicle control ($p < 0.01$).

The same thymic cell samples were analyzed for effects on specific T-cell subtypes (i.e., helper cells [CD4], suppressor cells [CD8], double-positive T cells [DP], double-negative T cells [DN]), and for treatment-induced changes in levels of apoptosis. Overall, all T cell subpopulations were decreased after Δ^9-THC exposure excepting CD4 cells.

The terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) method was used to detect DNA fragmentation generated during apoptosis, which was followed by flow cytometric analysis. The percentage of apoptotic fetal thymic cells increased with increasing dose of Δ^9-THC.

C. Pregnant mice pretreated with CB_1R or CB_2R antagonists, then subsequently given 50 mg/kg Δ^9-THC by i.p. injection on GD 16 showed at least partial blockage of the effects described above (see Figure 6 and Figure 7 below). These results support a role for both CB1 and CB2 receptors in Δ^9-THC-induced fetal thymic atrophy and apoptosis.

Figure 6. Effects of Pretreatment with CB1 or CB2 Receptor Antagonists on Thymic Cellularity of Δ9-THC-Treated Fetuses.

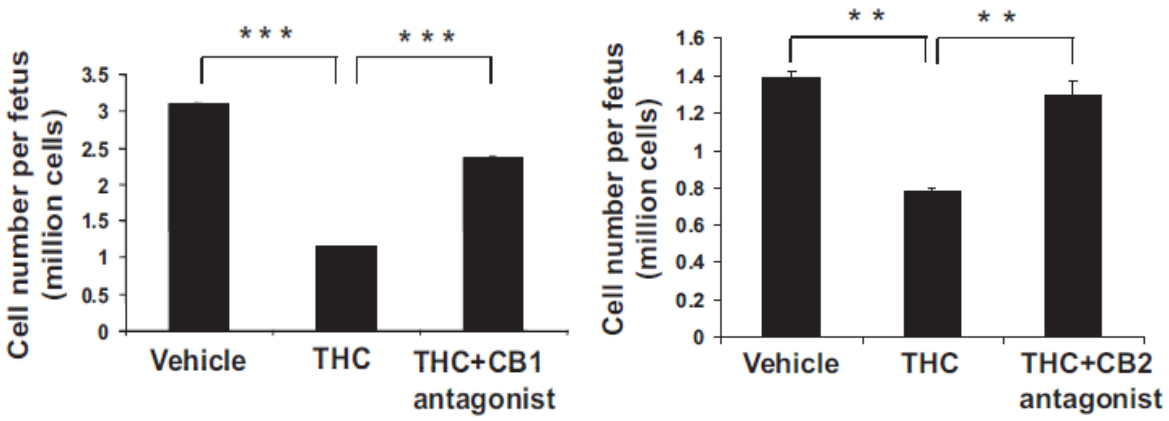


Figure 6. From Lombard et al. (2011) Groups of two GD16 pregnant C57BL/6 mice were pretreated with CB1 (SR141716A) or CB2 (AM630) receptor antagonists followed by injection with 50 mg/kg Δ^9-THC or the vehicle. On GD 17, thymi from all fetuses (average of 10/litter) were pooled and analyzed for viable cells/fetus as previously described. Data are presented as mean thymic cellularity, $\pm$ SEM. ***, $p < 0.0001$, one-way ANOVA. **, $p< 0.05$.

Percentages of apoptotic cells on GD 17 were determined by the TUNEL method and measured by flow cytometry as described for experiment number 2 above. The percentages of apoptotic cells were increased by Δ^9-THC-exposure on GD 16, while co-exposure to Δ^9-THC and CB1 or CB2 receptor antagonists resulted in near-control levels of apoptosis.

D. Experiment 4 was essentially a repeat of experiment 2 with additional, later cell harvest time-points. Thymi harvested from postnatal day (PND)1 pups, as well as GD 17 and GD18 fetuses, showed decreased cellularity (see **Figure 7** below) and increased apoptosis over control levels in offspring exposed to 50 mg/kg Δ^9-THC on GD16. Control offspring showed expected increases in thymic cellularity with increasing age up to the final assessment at PND 1.

Evidence for apoptosis on GD 18 and PND 1 came from the results of TUNEL staining, and the use of caspase-3/7 assays for detecting the activity of caspase proteolytic enzymes associated with apoptosis. The frequency of apoptotic cells on GD 18 increased from 10.64% in control fetal thymi to 72.3% in exposed fetal thymi (e.g., dams treated with 50 mg/kg Δ^9-THC on GD16). On PND 1, the corresponding frequencies were 42.6% for controls and 63.4% for prenatally exposed pups. Increases in caspase-3/7 activity with GD 16 Δ^9-THC-exposure were significant on both GD 18 ($p \leq 0.01$) and PND 1 ($p \leq 0.001$), by two-tailed unpaired Student's *t*-test.

Figure 7. Thymic Cell Number per Fetus/Pup on GD 17, GD 18, and PND 1 Following Δ9-THC-exposure on GD 16.

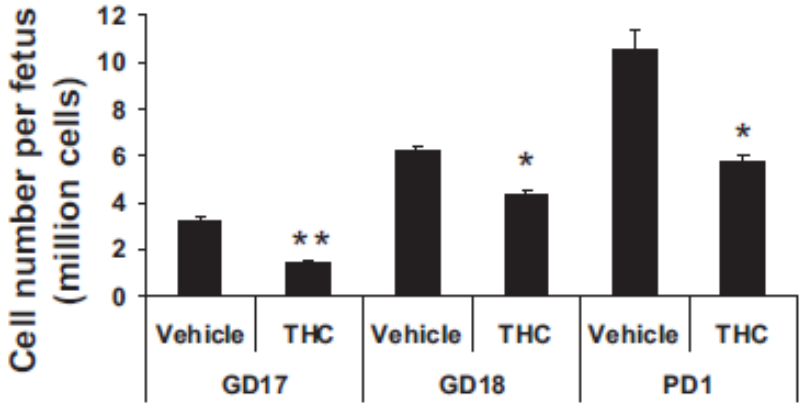


Figure 7. From Lombard et al. (2011). Groups of two pregnant C57BL/6 mice were treated with 50 mg/kg Δ^9-THC, or vehicle. Offspring thymi were harvested and pooled for determination of viable cellularity by trypan blue dye exclusion. Results are depicted as mean ± SEM; statistically significant difference between vehicle control and Δ^9-THC treatment group by two-tailed unpaired Student's *t* test (*, $p < 0.05$; **, $p < 0.01$).

E. Primary cultures of fetal thymic lobes (Fetal Thymic Organ Culture, or FTOC) were prepared from GD 16 mouse fetuses. FTOCs were exposed to Δ^9-THC concentrations of 0, 5, 10, or 20 µM in culture media for six days (with one change of media on culture day 3). Controls were exposed to the treatment vehicle, DMSO. Harvested cells were processed as for *in vivo* experiments described above.

Mean numbers of viable cells per organ decreased with increasing concentration of Δ^9-THC in culture media ($p < 0.001$ at each concentration compared to vehicle controls). At the same time, percentages of apoptotic cells increased with increasing Δ^9-THC concentration.

The results were taken to support a direct effect of Δ^9-THC on fetal thymic cells, rather than a consequence of maternal effects.

F. Postnatal immune function was tested in offspring exposed to 50 mg/kg Δ^9-THC on GD 16. At five weeks postnatal age, treated and control offspring were given footpad injections of 5 µg of HIV-1 p17/p24/gp120 emulsified in Complete Freund's Adjuvant (CFA). After an additional week, Δ^9-THC-exposed animals showed reduced serum levels of antibodies, and a lower proliferative response of lymphocytes from draining lymph nodes (draining LN) to an *in vitro* re-stimulation with the HIV strain (see Figure 8 below). Data were taken to indicate that gestational Δ^9-THC exposure reduced subsequent immune response to HIV antigens in young mice.

Figure 8. Effects on Postnatal Immune Response to HIV of Δ9-THC Exposure on GD 16.

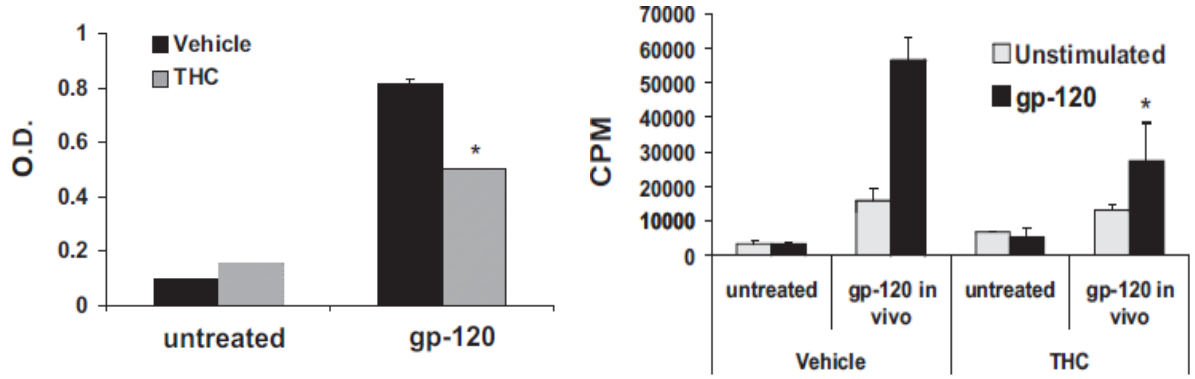


Figure 8. From Lombard (2011). Groups of two pregnant C57BL/6 mice were injected with 50 mg/kg Δ^9-THC or vehicle on GD16. Five weeks after the pups were born, they were injected in each rear footpad with 5 µg of HIV-1 p17/p24/gp120 emulsified in CFA. After 1 week, sera (left graph) and draining LNs (right graph) were collected. Sera were analyzed for the presence of HIV-1 p27/p24/gp120-specific IgG. Draining LN cells were left unstimulated or re-stimulated with 25 µg/ml of HIV-1 p17/p24/gp120 for 72 h. Data represent mean $\pm$ SEM of triplicate cultures. *, statistically significant differences, $p < 0.05$. On the left, one-way ANOVA, interaction, $p < 0.0005$; DMSO vehicle versus Δ^9-THC, $p < 0.017$; untreated versus gp120, $p < 0.0001$. On the right, one-way ANOVA, interaction not significant; DMSO versus Δ^9-THC, not significant; untreated versus gp120, $p = 0.0016$.

G. Thymi and spleens of one-week old mouse pups were harvested for analysis following Δ^9-THC treatment for four days prior to birth. Groups of two pregnant C57BL/6 mice were injected with 25 mg/kg Δ^9-THC on GD16 and 10 mg/ml Δ^9-THC every day thereafter until they delivered their litters (for a total of four injections, or 55 mg/kg Δ^9-THC). Controls were given vehicle only.

Mean numbers of viable cells per pup $\pm$ SEM showed the same pattern for both thymi and spleens of week-old pups: significant decreases in cellularity with Δ^9-THC-exposure by two-tailed unpaired Student's *t* test (thymus, $p = 0.0196$; spleen, $p = 0.0201$). Four major thymic cell subtypes (i.e., CD4, CD8, DP, DN) were all found to be significantly decreased in prenatally Δ^9-THC-exposed pups.

Harvested splenocytes from the same week-old pups described above, following prenatal exposure to Δ^9-THC, were cultured in the presence of mitogens and evaluated for proliferative response. Cultured cells were left unstimulated or stimulated for 48 hours with:

- Concanavalin A (Con A), a lectin known to stimulate mouse T-cell subsets.
- Lipopolysaccharide (LPS), regulates inflammation via induction of cytokine release, or
- Anti-CD3 mAb, a monoclonal antibody that binds to CD3 (cluster of differentiation 3) on the surface of T cells, suppressing T cell activation.

Uptake of [^{3}H]-thymidine during the final eight hours of culture was used to measure cell proliferation in response to each of the mitogens. The proliferative response was determined be significantly decreased in cultured splenocytes from Δ^9-THC-exposed pups compared to splenocytes from vehicle-exposed animals ($p \leq 0.01$ for each mitogen).

Taken together, these experiments were indicative of long-lasting alterations in the immune system following gestational exposure to Δ^9-THC. Individual experiments focused on documenting the role of thymic CB1 and CB2 receptors in signaling and induction of apoptosis, alterations in fetal thymic cell subpopulations, and the dose-dependency and lasting evidence of these effects through PND 1. Splenic, as well as thymic, cells showed effects of Δ^9-THC exposure. Depression of immune function in gestationally-exposed postnatal animals was evidenced by blunted responsiveness to HIV-1 p17/p24/gp120 and to the mitogens Con A, LPS, and anti-CD3 mAb.

Effects on Bone Growth

Although there is no direct evidence linking prenatal Δ^9-THC exposure to effects on linear bone growth, the available indirect evidence is important to understanding observed effects on general growth parameters reported in human and animal studies following prenatal exposures (as discussed by (Maccarrone et al. 2015b; Wasserman et al. 2015; Zuckerman et al. 1989b)). This indirect evidence includes studies of postnatal Δ^9-THC exposure in mice, as well as studies of genetically modified mice with knock-out mutations for CB1 and CB2 receptors. Each line of evidence listed below will be discussed in more detail in this section:

- The processes of bone growth and ossification begin prenatally and continue postnatally.
- The EC system has an important role in the processes of bone growth and remodeling at all stages of life, particularly during periods of rapid bone growth.
- EC receptor (i.e., CB1 and CB2 receptor) knock-out mutations in mice affect bone growth and remodeling.
- Δ^9-THC-exposure affects bone growth and remodeling *in vivo* and *in vitro*.

Normal Development and Growth of Long Bones

Starting prenatally and continuing through adolescence, growth and ossification of all long bones progresses via the same basic sequence (Cooper et al. 2013; DeSesso and Scialli 2018).

- Mesenchyme cells condense into a cartilaginous bone model

- A primary ossification center appears near the middle of each cartilage primordia.
- Secondary centers of ossification appear at either end of each long bone.
- Linear growth continues at the cartilaginous epiphyseal plates between the ossified areas.
- When the epiphyseal plates eventually fully ossify, the bone can no longer grow in length.

In mice, secondary ossification centers do not appear until after birth (Patton and Kaufman 1995), but in humans some secondary centers can be detected prenatally (Panattoni et al. 2000).

At the cellular level, the process of bone formation is outlined briefly as follows, from (Kronenberg 2003):

- Chondrocytes in the center of the cartilaginous bone model stop proliferating and hypertrophy.
- Hypertrophic chondrocytes act as master regulatory cells by:
  - Directing mineralization of cartilage matrix.
  - Attracting vascularization and chondroclasts.
  - Signaling perichondrial cells to differentiate into osteoblasts and produce bone matrix.
- Finally, the hypertrophic chondrocytes undergo apoptosis, leaving behind a cartilage matrix for population by bone-producing osteoblasts.

Bone growth proceeds via continued chondrocyte proliferation and waves of hypertrophy from the center towards each end.

Bone growth and remodeling in mice with CB1 or CB2 receptor knock-out mutations.

A role for the EC system in normal bone growth and remodeling has been supported by studies of mice expressing knock-out mutations for CB1 and CB2 receptors (Bab and Zimmer 2008; Bab et al. 2009; Maccarrone et al. 2015b; Ofek et al. 2006; Wasserman et al. 2015). The review article by Bab et al. (2009) describes the phenotypes of mutant crosses as follows:

- CB1 knockout
  - $CD1^{CB1-/-}$ (back-cross to CD1): Female mice were found to have essentially normal bone mass, while males exhibited higher bone mass. Once sexually mature, bone resorption and formation were normal in both sexes. These findings were taken to indicate that the

higher bone mass in males is acquired during development, at the time peak bone mass is determined.

- C57$^{CB1-/-}$ (back-cross to C57BL/6J): Both male and female mice were found to have low bone mass accompanied by increased osteoclast counts and a decreased rate of bone formation.

- CB2 knockout:
  - CNR2$^{-/-}$ mutation phenotype: Young animals of both sexes acquire normal peak bone mass. Later in life, these animals displayed enhanced age-related bone loss. In similarity to human osteoporosis, a high bone turn-over with increases in both resorption and formation results in a net loss of bone mass.

In a study described in detail below (Wasserman et al. 2015), untreated mice of a CB2 knockout strain developed longer femora and vertebral bodies than WT controls. Figure 9 below shows femoral growth data; the companion figure for vertebra is not shown here.

Effects of Δ^9-THC-exposure on bone growth and remodeling in vivo and in vitro.

Wasserman et al. (2015) studied the EC system and effects of Δ^9-THC on chondrocyte differentiation *in vitro*, and on linear bone growth *in vivo*. While the *in vitro* experiments involved primary cells from wild type (WT) mice, the experiments performed *in vivo* compared effects of Δ^9-THC on WT versus CB1 and CB2 "knock-out" mice.

An inhibitory effect of Δ^9-THC on chondrocyte differentiation was seen in the *in vitro* experiment. Primary cultures of chondrocytes from PND 4 WT mouse pups showed biochemical and morphological differentiation over a 13-day period. Hypertrophic cells developed and began to form nodules, in association with increased expression of mRNA transcripts for cannabinoid receptors and diacylglycerol lipases. Addition of 10^{-11}M to 10^{-7}M Δ^9-THC to culture media was found to inhibit nodule formation in a concentration-dependent manner. These results were taken to support a direct effect of cannabinoid signaling on chondrocyte differentiation and nodule formation.

Skeletal development *in vivo* was analyzed in CB-deficient "knock-out" mice, as well as in animals exposed to Δ^9-THC during a period of rapid skeletal growth. C57BL/6J mice were bred with mice having deletion mutations for CB1 or CB2 receptors.

- C57BL/6J *Cnr1−/−* (CB1 deletion on a C57BL/6J background)
- C57BL/6J *Cnr2−/−* (CB2 deletion on a C57BL/6J background)
- C57BL/6J *Cnr1−/− Cnr2−/−* (double CB1/CB2 deletion on a C57BL/6J background)
- C57BL/6J (WT controls)

Δ^9-THC was given daily at a dose of 5 mg/kg-day by i.p. injection to young mice between the ages of five and 11 postnatal weeks. Length of femora and L3 vertebral bodies from 11-week old mice were determined using microcomputed tomography (µCT). In order to compare any effects on linear bone growth with the proportion of total body weight due to fat accumulation, intrascapular fat pads from each animal were dissected out and weighed at the time of sacrifice.

In the absence of Δ^9-THC, either or both of the CB receptor deletion mutations tended to enhance elongation of skeletal elements relative to WT animals. Δ^9-THC exposure was associated with decreased femoral length in female pups of the WT or CB2$^{-/-}$ genetic groups (see Figure 10 below). Femur length in CB1$^{-/-}$ or double mutant mice was unaffected, suggesting Δ^9-THC interacts with the CB1 receptor in affecting bone growth. While less visually dramatic, vertebral body length of L3 from the same pups showed the same pattern of growth associated with Δ^9-THC and/or CB receptor mutations (figure not shown here).

Figure 9. Femur Length in Female GD 11 Pups with or without Δ^9-THC Exposure.

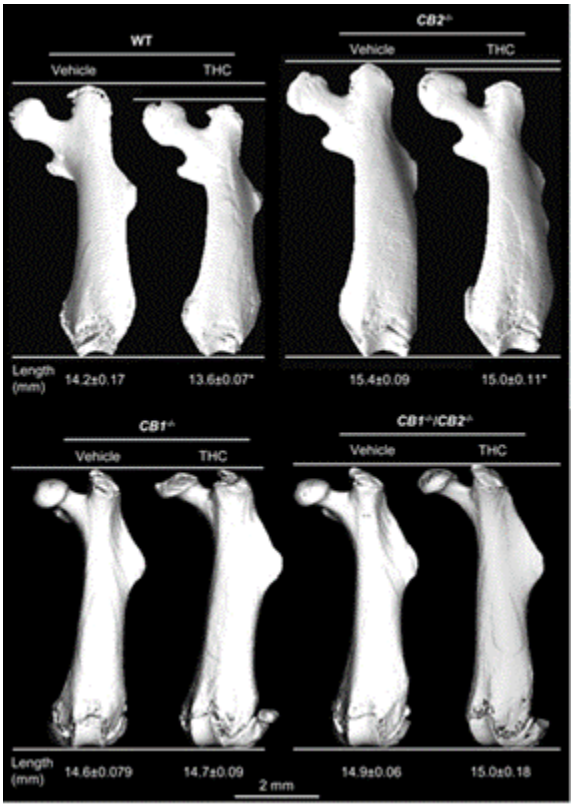


Figure 9. Δ^9-THC attenuates female femoral length in a CB1-dependent manner. Data are mean ± SE obtained in 7–12 mice per condition. *p < 0.05 vs. vehicle (Wasserman et al. 2015).

The weights of intrascapular fat pads did not differ significantly with exposure to Δ^9-THC, regardless of sex or genotype of pups. Total body weights of male pups over the treatment period also did not show significant effects of Δ^9-THC regardless of genotype.

For female pups, body weights of Δ^9-THC-exposed animals were significantly decreased ($p < 0.05$) compared to controls for WT and *Cnr2−/−* animals during the treatment period. No treatment effects on body weight were seen for *Cnr2−/−* or *Cnr1−/− Cnr2−/−* pups (see Figure 10 below).

Figure 10. Effects of Δ9-THC on Body Weights and Fat Pad Weight of Female Mouse pups of the WT, Cnr2−/−, Cnr1−/−, or Cnr1−/− Cnr2−/− genotypes.

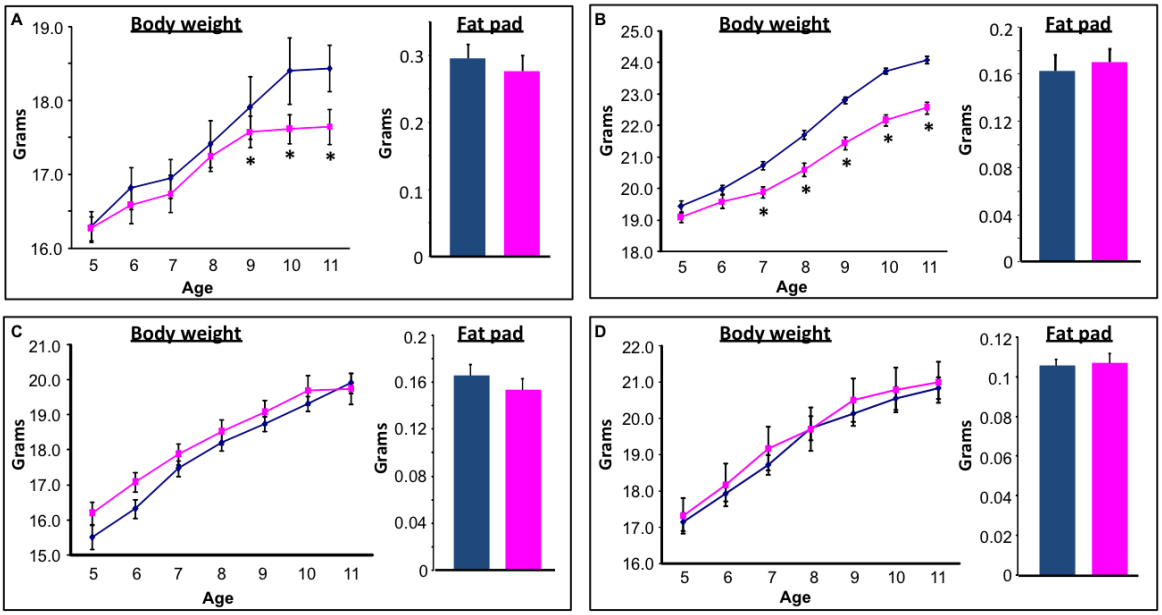


Figure 10. A. Female WT pups, B. Female *Cnr2−/−* pups, C. Female *Cnr1−/−* pups, D. Female *Cnr1−/− Cnr2−/−* pups. Data points represent mean ± SE values for 7-12 animals per condition. *$p < 0.05$ (Wasserman et al. 2015).

In combination with bone growth data, the body and fat pad weight results were considered to indicate that observed reductions in body weight of female mice were related to bone growth rather than fat deposition. Δ^9-THC was found to affect body weight and bone growth only in mice having functional CB1 receptors (WT or *Cnr2−/−*).

While direct evidence is lacking, these results may have implications for understanding observed effects of Δ^9-THC on growth outcomes reported in human and animal studies.

Studies on Neurodevelopment

Rodents and Non-Human Primates

A number of studies have been conducted in animals to investigate the neurodevelopmental effects of prenatal or perinatal exposure to either cannabis smoke,

cannabis extracts, or Δ^9-THC. Most of these studies have been conducted in rats, although three studies were conducted in mice, and one study was conducted in Rhesus monkeys. Exposure in studies was preconception or during the prenatal period (3 via the inhalation route, 11 via the oral route and 6 via parenteral routes) and 23 studies included a postnatal component of exposure (16 via the oral route and 7 via parenteral routes). Descriptions of the study designs and findings are provided in the tables in **Appendix 4.** Animal Neurodevelopmental Toxicity Studies).

Some studies conducted perinatally in rodents may be directly relevant to human prenatal exposures, because the developmental stage of the neurological structure subject to the exposure in the postnatal period in some laboratory animal species may correspond to the developmental stage occurring during gestation in humans. Using a database originally assembled by Finlay and Darlington, (1995), the equivalent post conception (PC) dates across mammalian species have been estimated. This online resource (http://www.translatingtime.net/) can be used to understand roughly equivalent neurodevelopmental phases across species used in animal testing and humans. Information from this publicly accessible website indicates that the early postnatal period in rodents can correlate to prenatal periods in the human for several events of neurogenesis (Clancy et al. 2007; Workman et al. 2013)

The studies outlined below and summarized in Tables in Appendix 4. Animal Neurodevelopmental Toxicity Studies have evaluated a variety of neurodevelopmental effects, ranging from endpoints related to spontaneous motor activity, stereotyped behaviors, behavioral response to novelty, sex behavior, learning and memory, emotionality, and vulnerability to drug abuse. Other endpoints assessed include alterations in gene expression and protein levels in specific brain regions. Some of these studies also reported on general somatic developmental endpoints, and these findings have been presented above and in Appendix 3. Animal Developmental Toxicity Studies: Somatic Development

Appendix Table 3.1: in vitro and in vivo Animal Studies: Early Embryo Development and Implantation

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In Appendix 4. Animal Neurodevelopmental Toxicity Studies, the animal neurodevelopmental toxicity studies, are presented in separate Appendix Tables 4 covering cannabis exposure by different routes and different windows of exposure (pre-conceptual, prenatal and perinatal).

An overview of the findings from these neurodevelopmental studies is provided below, in the following broad categories: locomotor and exploratory activity, cognitive function, emotionality, drug sensitivity later in life, other behavioral effects, other neurodevelopmental effects.

Locomotor and exploratory activity

Various behavioral studies examined the motor behaviors and exploratory activities in adulthood and their relationship to neurochemical studies after perinatal Δ^9-THC exposure in rats. Altered spontaneous locomotor and exploratory behaviors, as well as impairments in social interactions and behavioral responses to novelty were investigated. Prenatal and perinatal exposure to Δ^9-THC (GD 5 - PND 24) resulted in long-term effects on locomotor and exploratory behaviors, including increased locomotor activity, sniffing, and rearing (Rubio et al. 1995), along with sexually dimorphic behavioral and endocrine alterations in adaptive responses to stressors such as novelty or place-preference testing (Rubio et al. 1995; Rubio et al. 1998). Additionally, female offspring exposed to Δ^9-THC showed greater locomotor activity than controls, and male offspring showed increased exploratory behavior in a plus-maze paradigm (Rubio et al. 1995). Overall, the observed effects of perinatal exposure to Δ^9-THC or a cannabis extract suggest that motor behaviors are altered, as well as HPA activity (Navarro et al. 1994; Navarro et al. 1995; Navarro et al. 1996; Rubio et al. 1995; Rubio et al. 1998).

The effect of perinatal exposure to Δ^9-THC (GD 5 –PND 24) on psychomotor behavior and neuroendocrine profile was evaluated in rats (Moreno et al. 2003). Δ^9-THC exposed animals were reported to be less active in the open field tests, with increased time spent in immobility (defined as the time spent by the animals in absolute quietness). Presynaptic dopamine (D_2) receptor sensitivity to the agonists apomorphine and quinpirole was also evaluated. In Δ^9-THC exposed offspring challenged with apomorphine, there was an increase in the immobility of males, but not females, compared to controls. In Δ^9-THC exposed offspring challenged with quinpirole, there was an initial increase in immobility observed during the first five minutes in males compared to controls, followed by a decrease in immobility, with no effects in females, compared to controls.

In a subsequent study with similar perinatal exposure to Δ^9-THC, decreased locomotor activity was observed in adult male offspring at Δ^9-THC doses of 0.1 and 2 mg/kg and in females at doses of 0.1 and 0.5 mg/kg, while the highest Δ^9-THC dose of 2 mg/kg resulted in a decrease in locomotor activity in females, which the authors interpreted as a lack of habituation (Moreno et al. 2005). The perinatal Δ^9-THC exposure generally induced an increase in immobility behavior in both sexes and decreased exploratory behavior, although the decrease in exploratory behavior was statistically significant only in females exposed to 0.1 and 0.5 mg/kg Δ^9-THC, with an increase observed in females exposed to the highest dose of 2 mg/kg. These sex-dimorphic behavioral effects of

perinatal exposure to Δ^9-THC were accompanied by sex-dimorphic alterations in plasma corticosterone levels (reduced levels in males and increased levels in females). The authors speculate that the changes in HPA activity, as indicated by altered plasma corticosterone levels, could mediate these effects. Other studies reported no consistent change in locomotor activity (Brake et al. 1987; Fried 1976; Navarro et al. 1995; Trezza et al. 2008; Vardaris et al. 1976). An earlier study showed a dampened locomotor response to amphetamine in both male and female, though females were more active overall but both sexes were sensitive to the locomotor altering effects of Δ^9-THC (Silva et al. 2012). A recent study reported parental Δ^9-THC exposure resulted in decreased initial movements in female, but not male, offspring (Szutorisz et al. 2016) while in a study in offspring of male rats exposed to Δ^9-THC preconceptionally, a small but significant increase in habituation of locomotor activity was observed in offspring at 18 – 20 weeks of age, but not at week 5 (relative to control animals) (Levin et al. 2019).

Three studies exposed pregnant rats to cannabis smoke and each reported decreased activity in the open field, which is regarded as a test of general activity and exploratory drive in the offspring (Charlebois and Fried 1980; Fried 1976; Fried and Charlebois 1979).

Cognitive function

A number of studies investigated the effects of prenatal exposure to Δ^9-THC on a variety of cognitive endpoints, including spontaneous alternation, shock avoidance, rotorod behavior, maze learning and passive avoidance learning (Abel, 1984; Abel et al. 1990a; Abel and Subramanian 1990b; Gianutsos and Abbatiello 1972) with individual studies focusing only on some of these endpoints. No treatment -related effects on these endpoints were observed in these studies. However, in a rat study of prenatal exposure on GD 8-11 to a cannabis extract, offspring of the exposed dams committed significantly more errors and required more time to complete a maze task, when animals were tested in adulthood (Gianutsos and Abbatiello 1972). Based on this study, there appeared to be a critical period during embryonic development when cells or tissues of the developing fetus were prone to damage in those areas of the brain related to maze learning.

In a study of female monkeys treated chronically (for over two years prior to conception and throughout pregnancy and lactation) with Δ^9-THC, altered regulation of visual attention was observed in offspring. Animals failed to limit the time spent on visual attention when presented with a novel stimulus (Golub et al. 1981). In control animals, neither novelty nor complexity influenced the duration of attention while offspring exposed to Δ^9-THC directed more attention at novel stimuli than at familiar stimuli (both simple and complex).

Effects on learning and spatial discrimination of postnatal exposure to Δ^9-THC on PND 4-14 were evaluated in adult rats, beginning on PND 56, using a two-component food-

motivated double Y-maze test (O'Shea and Mallet 2005). The authors characterized the PND 4-14 in the rat as a major period of synaptogenesis that corresponds to the third trimester in humans. Postnatal Δ^9-THC exposure adversely affected learning ability, as indicated by slower acquisition of the delayed alternation component of the double Y-maze task. Δ^9-THC-exposed rats committed significantly more errors, and required significantly longer to obtain 80% correct over two consecutive days in the delayed alternation task. No exposure-related effects on spatial discrimination were observed. According to the authors, the effects on learning observed in this study were likely due to an alteration of cognitive ability that impaired working memory function.

Effects of perinatal Δ^9-THC exposure during GD 15-PND 9 on two forms of memory, namely short-term social memory and a long-lasting aversive memory (social discrimination tests and inhibitory avoidance) were evaluated in adult male rats beginning on PND 80 (Campolongo et al 2007). In the social discrimination tests of short-term memory, the ability of adult male rats to discriminate between a novel and a familiar rat was statistically significantly impaired in rats exposed perinatally to Δ^9-THC, compared to controls. And in the inhibitory avoidance test of long-lasting aversive memory the ability to remember the task when the trial was repeated 24 hours late was statistically significantly impaired in rats exposed perinatally to Δ^9-THC, compared to controls. Basal extracellular cortical levels of glutamate and norepinephrine were measured, and found to be significantly decreased in rats exposed perinatally to Δ^9-THC, compared to controls. These authors also used microarray technology to investigate global gene expression in the prefrontal cortex (PFC) of control and Δ^9-THC-exposed animals on PND 80. Perinatal Δ^9-THC exposure was associated with decreased expression in the PFC of genes related to myelination, and increased expression of genes involved in apoptosis. In additional gene expression microarray studies conducted by these researchers, 141 genes (out of 10480 screened = 1.3%) were determined to be differentially expressed in perinatal Δ^9-THC exposed rats, with the majority of Δ^9-THC-responsive genes falling into the following gene ontology categories: development, neurogenesis, myelination, synaptic transmission, response to stress, signal transduction, cell signaling, metabolism and apoptosis (Campolongo et al. 2007; Campolongo et al. 2009; Campolongo et al. 2011).

Prenatal Δ^9-THC had no effect on acquisition, but, impaired consolidation during retention testing and reversal learning was noted in exposed offspring during the adolescent and juvenile periods (Silva et al. 2012). In a study in offspring of male rats exposed to Δ^9-THC preconceptionally (paternal), long-lasting impairment in attentional performance, as measured by an operant visual attention task, was observed in adulthood (Levin et al. 2019).

Emotionality

CB_1Rs are highly expressed in brain areas involved in the modulation of emotionality and thus the effects of perinatal Δ^9-THC exposure to rats during GD 15-PND 9 on several measures of emotional reactivity were investigated in exposed male offspring at PND 12, 35, and 80 (Campolongo et al. 2009; Trezza et al. 2008). When animals were placed in isolation on PND 12, the rats perinatally exposed to Δ^9-THC made significantly more ultrasonic vocalizations than did controls. At PND 35, rats perinatally exposed to Δ^9-THC displayed inhibited social interaction and play behavior, compared to controls. And when animals were assessed on an elevated plus maze on PND 80, the rats perinatally exposed to Δ^9-THC exhibited increased anxiogenic behavior, namely, spending less time on the open arms of the maze, compared to controls. In a study evaluating anxiety, two measures of anxiety in rats were examined - open field behavior and the forced swim test. After perinatal exposure to Δ^9-THC, via subcutaneous injection (2 mg/kg) male offspring showed decreased time in the inner part of the open field, one measure of increased anxiety. Increased social interaction compared to controls with no differences from controls in the forced-swim test was also reported (Newsom and Kelly 2008).

Drug sensitivity later in life

In animal studies perinatal cannabinoid exposure affects the ontogeny of the central nervous system altering neurotransmitter systems (Fernandez-Ruiz et al. 2000; Fernandez-Ruiz et al. 1999). These changes include an enhancement of the rewarding properties of drugs of abuse such as opiates. CB_1 receptors and μ-opioid receptors are distributed in many of the same areas in the brain, such as the periaqueductal gray, locus coeruleus, ventral tegmental area (VTA), nucleus accumbens (NAc), PFC, central amygdala, bed nucleus of stria terminalis (BNST), caudate putamen (CP), substantia nigra, dorsal hippocampus, raphe nuclei, and medial basal hypothalamus. Both the frequent co-localization of these receptors and the extent of this overlapping expression is thought to provide a basis for interactions between the opioid and cannabinoid systems in reward and addiction withdrawal (Wiese and Wilson-Poe 2018).

A number of studies examined animal behavior for enhanced adult experience or increase frequency of use of opiates or alcohol after pre- or perinatal exposure to Δ^9-THC. Adult animals exposed to Δ^9-THC in utero or via lactation, were reported to have an increased rate of acquisition of morphine self-administration and or enhanced sensitivity towards the rewarding effects of morphine, such as spending more time in morphine-paired compartment than in a saline-paired compartment (Navarro et al. 1994; Navarro et al. 1995; Navarro et al. 1996; Rubio et al. 1995; Rubio et al. 1998; Vela et al. 1998; Singh et al. 2006; Singh et al. 2006; Singh et al. 2006). The susceptibility to reinforcing effects of morphine in adulthood, after perinatal Δ^9-THC exposure has been studied with intravenous morphine self-administration (Vela et al. 1998). In this study, perinatal (GD 5 - PND 24) exposure to Δ^9-THC was reported to alter the susceptibility to morphine reinforcing effects in adult female offspring, along with changes in μ-opioid

receptor binding in several brain regions. This effect was not observed in the male offspring. Changes in μ-opioid receptor binding differed regionally in male and female offspring, with Δ^9-THC-exposed males exhibiting a lower density than controls in the caudate-putamen area as well as in the amygdala (posteromedial cortical nucleus), while female offspring had higher density of these receptors than controls in the prefrontal cortex, the hippocampus (CA3 area), the amygdala (posteromedial cortical nucleus), the ventral tegmental area and the periaqueductal grey matter, but lower binding than controls in the lateral amygdala. Additionally, the reinforcing value of food seems to be independent of sex and perinatal exposure to Δ^9-THC in this study. The development of tolerance to morphine as well as the dependence to morphine that was observed suggests that the reinforcing value of morphine was reported to be greater than food in Δ^9-THC-treated female adult offspring. (Vela et al. 1998). Increased sensitivity to the reinforcing properties of morphine were observed in adult males perinatally exposed to 1 or 5, but not 20 mg/kg-day Δ^9-THC from GD 5-PND 24 (Rubio et al 1998).

In one study using a progressive ratio (PR) schedule in which the response requirement increased for operant food- and morphine-reinforced behavior, female offspring respond more intensely than male. The data indicate that, when rats are forced to work harder for the reward, the exposure to Δ^9-THC does not influence the response to both reinforcers. Neurochemical analysis showed that the activity of limbic dopaminergic neurons, prior to morphine self-administration, was higher in females than males, and that the perinatal Δ^9-THC reduced the activity of these neurons only in females, although there was no influence on morphine vulnerability (Gonzalez et al. 2003). Another study following perinatal exposures Δ^9-THC did not report effects on ethanol self-administration (Economidou et al. 2007). In the offspring of adolescent females exposed to Δ^9-THC, an anhedonic phenotype with lower sensitivity to natural rewards and susceptibility to addictive behaviors was reported (Pitsilis et al. 2017).

Singh et al. (2006) observed that the rewarding properties of heroin given to male rats at 8 weeks of age, were enhanced by neonatal Δ^9-THC exposure via intraperitoneal (i.p.) injection during PND 4-14, and that a marker of heroin-induced neuronal activation (i.e., Fos-immunoreactivity (Fos-IR)) was increased in the dorsomedial caudate putamen by perinatal Δ^9-THC exposure (Singh et al. 2006). Similarly, enduring effects of perinatal Δ^9-THC exposure via intravenous (i.v.) injection during GD 5 - PND 2 were observed in adult male rat offspring, measured as increased heroin-seeking behavior during mild stress and drug extinction, and changes in preproenkephalin (PENK) mRNA levels in the nucleus accumbens (NAc) (decreased expression compared to controls at PND 2, and increased expression compared to controls in adulthood). The authors state that changes in PENK expression are relevant to both drug motivation/reward and the stress response (Spano et al. 2007). Maternal cannabis use was reported to decrease DRD2 mRNA levels in the NAc in human fetuses collected after elective abortions, as also in

rats prenatally exposed to Δ^9-THC along with enhanced opiate reward conditioned place sensitivity (DiNieri et al. 2011).

Adult rat F1 offspring whose parents were exposed preconceptionally to Δ^9-THC, but were themselves unexposed to Δ^9-THC displayed increased effort (i.e., increased lever pressing) to self-administer heroin, along with enhanced stereotypy during the stressful period of acute heroin withdrawal, compared to controls (Szutorisz et al. 2014). Increases in mRNA for expression of cannabinoid, dopamine, and glutamatergic receptor genes (Cnr1, Grin2A, Gria2) were observed in the ventral striatum, a key component of the neuronal circuitry mediating compulsive behaviors and reward sensitivity, at PND 35 in offspring of parents preconceptionally exposed to Δ^9-THC. Decreases in mRNA for expression of these same genes and other genes associated with these same receptors (Cnr1, Grin2A, Grin1, Gria2, Gria1) were observed in the dorsal striatum at PND 62 in offspring of parents preconceptionally exposed to Δ^9-THC. Also at PND 62 these animals had decreased levels of NMDA receptor proteins (GluN1, GluN2B), decreased NMDA receptor binding, and increased LTD in the dorsal striatum, compared to controls. Similar changes in striatal gene expression were reported in in offspring of rats preconceptionally exposed to Δ^9-THC by Szutorisz et al (2016).

Other behavioral effects

Increase in the frequency and time spent grooming was observed in both adult male and female rats after oral exposure to Δ^9-THC during GD 5-PND 24 (Navarro et al. 1994). A similar perinatal exposure to a Hashish extract resulted in an altered pattern of approach of adult male rats toward sexually receptive females, as well as decreased dopamine in the adult male limbic forebrain, with no such effects observed in females (Navarro et al. 1996).

Hutchings et al. (1991) reported no effects of orally administered Δ^9-THC during the prenatal period on auditory startle at PND 57–60 in both male and female rats, similar to the findings with synthetic Δ^9-THC analogues, where reflex reactivity to environmental stimuli in the offspring were unaffected (Campolongo et al., 2011).

Other neurodevelopmental effects

Effects of perinatal Δ^9-THC on the catecholamine system has been investigated in multiple studies in rats. In one study, a significant increase in the maximum binding capacity (B_{max}) of α_1-adrenergic receptors in the cerebral cortex was observed at PND 20, but not on PND 40 or 60 in rats exposed perinatally via oral administration during gestation through PDN 20 (Walters and Car 1988). No effects of perinatal Δ^9-THC were observed on the B_{max} of D_2 dopaminergic receptors in the striatum at any age. Other studies have examined neuronal expression of tyrosine hydroxylase (TH), a key enzyme involved in the synthesis of dopamine and other catecholamines in rats exposed to Δ^9-THC prenatally (Bonnin et al. 1995; Bonnin et al. 1996). TH is thought to play an

important role in neuronal developmental processes such as axonal guidance, neuronal recognition and synaptogenesis. Two studies observed that prenatal exposure to Δ^9-THC from GD 5 – to sacrifice on GD 14 or GD 16 resulted in increased TH gene expression and enzyme activity (or protein levels, in the case of Bonnin et al 1996) in the brain compared to controls on GD 14, which normalized to control levels on GD 16 (Bonnin et al. 1995; Bonnin et al. 1996). The study of Bonin et al (1996) found that with continued Δ^9-THC exposure, brain levels of TH mRNA were increased in females, but not in males at GD 21, compared to controls. No treatment-related differences in TH gene expression were observed at later time points (PND 1, 5), however. Concordance between dopamine and norepinephrine levels and TH enzyme activity was not observed at all timepoints in these studies, suggesting that Δ^9-THC may have an effect on fetal TH gene expression, but not on catecholamine synthesis (Bonnin et al. 1995; Bonnin et al. 1996).

The effects of prenatal Δ^9-THC exposure (GD 12.5- GD 16.5) was investigated in a series of studies in mice (de Salas-Quiroga et al. 2015). Effects on cortical neuron development and CB_1R protein and receptor binding levels were studied in mice with normal expression of CB_1R (wild type; $CB_1^{+/+}$), and effects on corticospinal motor neuron-dependent function (i.e., skilled motor function assessed in skilled-reaching and staircase tests) and seizure susceptibility were investigated in mice with either no expression of CB_1R (Stop-CB_1; $CB_1^{-/-}$), restored CB_1R expression in all tissues on a Stop-CB_1 background (CB_1-RS), restored CB_1R expression only in glutamatergic cortical neurons (Glu-CB_1-RS), or restored CB_1R expression only in forebrain GABAergic neurons (GABA-CB_1-RS) (de Salas-Quiroga et al. 2015). Prenatal Δ^9-THC exposure of wild type mice affected cortical neuron development, reducing the number of subcerebral projection neurons at PND 20, and decreasing fetal (GD 17.5) but not PND 2.5 brain levels of CB_1R (i.e., protein and receptor binding). Prenatal Δ^9-THC exposure affected corticospinal motor neuron-dependent function in PND 60 mice expressing CB_1R in glutamatergic cortical neurons ($CB_1^{+/-}$, CB_1-RS, Glu-CB_1-RS); no effect of prenatal Δ^9-THC on skilled motor function was seen in mice lacking CB_1R expression in glutamatergic cortical neurons (Stop-CB_1, GABA-CB_1-RS). Prenatal Δ^9-THC exposure increased susceptibility to pentylenetetrazole (PTZ)-induced seizures in PND 60 mice expressing CB_1R ($CB_1^{+/-}$, CB_1-RS), but not in mice lacking expression (Stop-CB_1), with intermediate effects observed in mice expressing CB_1R only in glutamatergic neurons (Glu-CB_1-RS) or GABAergic neurons (GABA-CB_1-RS). The authors postulate that these effects are the result of disruption of physiological CB_1R signaling during a critical period of cortical neuron development, prior to the establishment of synaptic neuronal activity (de Salas-Quiroga et al. 2015).

The effect of prenatal (GD 5-21) Δ^9-THC exposure in rats on gene expression of neural cell adhesion molecule (N-CAM), also known as L1, was studied in fetal (GD 21) rat brain (Gomez et al. 2003). N-CAM is thought to be involved in cell proliferation and

migration, neuritic elongation and guidance, synaptogenesis and myelinogenesis. Prenatal Δ^9-THC exposure increased N-CAM gene expression in most of the white matter brain regions analyzed, including in the transverse commissural tracts such as the fimbria, the stria terminalis, the stria medullaris and the corpus callosum; increases were also observed in glial cells. Modest increases in N-CAM gene expression were observed in the habenula and the septum nuclei, areas implicated in addiction pathways. The authors noted that CB_1Rs are present in the same areas of the fetal brain in which increased N-CAM gene expression was observed. In controls, basal levels of N-CAM gene expression were generally higher in male, compared to female fetal brain, although the increases were not statistically significant. With prenatal Δ^9-THC exposure, N-CAM gene expression was significantly higher in the male fetal brain, compared to female fetal brain (Gomez et al. 2003).

In another study, using immunohistochemical analysis, the cerebella of rats perinatally exposed to Δ^9-THC during GD 5-PND 20 were examined at PND 20, 30 or 70 for expression of two different glutamate transporter subtypes, i.e., GLAST, which is present in astroglial cells, and EAAC1, which is present in Purkinje neurons (Suárez et al. 2004). GLAST levels were significantly reduced in the cerebella of rats perinatally exposed to Δ^9-THC on PND 20, 30 and 70, compared to controls, and EAAC1 levels were significantly reduced on PND 20 and 30.

Perinatal Δ^9-THC exposure during GD 15- PND 9 resulted in changes in the hippocampus of adult male rat offspring, as assessed on PND 90 (Beggiato et al 2017). In this study, binding to CB_1Rs was measured as B_{max} in hippocampal slices from 90-day-old male rats. Perinatal Δ^9-THC exposure resulted in a significant decrease in B_{max}, compared to controls. GABAergic signaling was also evaluated in these hippocampal slices, as basal and potassium (K^+)-evoked GABA outflow and update, and as K^+-evoked GABA outflow in response to a challenge with Δ^9-THC. Perinatal Δ^9-THC exposure resulted in significant decreases in basal GABA outflow and uptake, significant decreases in K^+-evoked GABA outflow and uptake, and a significant decrease in K^+-evoked GABA outflow in response to a Δ^9-THC challenge, compared to controls. These effects of perinatal Δ^9-THC exposure on GABAergic signaling in hippocampal slices were significantly blocked when the selective CB_1R antagonist SR141716A was added, confirming that CB_1R is the target affected by perinatal Δ^9-THC exposure. As noted by the authors, disturbances in GABAergic signaling may result in anomalous information processing, leading to cognitive impairments and behavioral consequences (Beggiato et al. 2017).

In other studies conducted using hippocampal slices from 40-day-old male rats exposed via a similar perinatal Δ^9-THC protocol (during GD 15- PND 9), elements of glutamatergic signaling were investigated (Castaldo et al. 2010). Perinatal Δ^9-THC exposure resulted in a significant decrease in the levels of two glutamate transporters,

namely glutamate transporter 1 (GLT1) and glutamate/aspartate transporter (GLAST), in hippocampal synaptosomes, compared to controls. Glutamatergic signaling was measured in the hippocampal slices as glutamate uptake and as glutamate release in response to a challenge with Δ^9-THC. Perinatal Δ^9-THC exposure resulted in significant decreases in glutamate uptake and in a significant decrease in glutamate release in response to a Δ^9-THC challenge, compared to controls.

Epigenetic Changes following prenatal and perinatal Δ^9-THC exposure are discussed in the following section (**F),** with detail study descriptions in Appendix Tables in Appendix 5, covering human and animal exposure in different age windows early in life.

Zebrafish

Four studies in zebrafish assessed neurodevelopmental effects, as well as some morphological endpoints (Achenbach et al. 2018; Ahmed et al. 2018; Akhtar et al. 2013; Carty et al. 2018). A visual motor response test, which is a behavioral test that relies on the integrity of the central and peripheral nervous systems, including the visual system, and on normal locomotor and skeletal system development, was used in three of these studies (Achenbach et al., 2018; Akhtar et al. 2013; Carty et al. 2018), The design and results of these studies are presented in Appendix Table 4.6. Zebrafish larvae start to express CB_1R by 24 hours post-fertilization (hpf). By 48 hpf, expression of CB_1R within the zebrafish CNS is widespread, and occurs within the preoptic area, telencephalon, hypothalamus, tegmentum, and anterior hindbrain.

In one study employing a visual motor response test by Carty et al. (2018), the movement of zebrafish larvae during dark conditions was measured and compared with movement during bright light conditions. Assessment of locomotor activity in response to the transition from light to dark was conducted only with larvae healthy enough to be included in a behavioral analysis, i.e., able to respond to touch. In this study, larvae were exposed to 0.3, 0.6, 1.25 or 2.5 mg/L Δ^9-THC. Exposure to the two lowest concentrations of Δ^9-THC (0.3 and 0.6 mg/L) resulted in significant hyperlocomoter activity in dark periods compared to controls, however, exposure to a higher concentration of Δ^9-THC (1.25 mg/L) resulted in hypolocomoter activity in dark periods compared with controls. Typically, zebrafish larvae have increased duration of movement (hyperlocomoter) during dark periods and decreased (hypolocomoter) behavior during bright conditions. A reversal in the characteristic light: dark behavior by larval zebrafish has been interpreted as an expression of stress or anxiety (Carty et al., 2018). The expression of select neurogenic genes (e.g., dazl, c-fos, vasa, sox2, sox3, sox9a, bdnf, reln, krit1, and cnr1) was also examined, following exposure to Δ^9-THC. Δ^9-THC upregulated c-fos in a concentration dependent manner in zebrafish. A non-dose dependent increase in the expression of dazl and a decrease in the expression of vasa

were also observed after Δ^9-THC exposure. No statistically significant differential expression of the other genes compared with control was detected (Carty et al. 2018).

In a study employing a modified visual motor response test by Akhtar et al. (2013), the effect of Δ^9-THC on the total distance moved in the basal, challenge, and recovery phases was investigated, following both acute and chronic exposures. Acute exposure was characterized as a 1–12-h exposure starting at 108 hpf while chronic exposure was characterized as a 96-h exposure starting at 24 hpf. After acute exposure (1, 4, or 12 h), a biphasic pattern was observed with increasing dose and time. Specifically, an increase in activity above that of control was observed at lower concentrations (0.6 and 1.2 mg/ml) at all time points, while a relative decrease in activity was observed over time at higher concentrations (2.4 and 3.4 mg/mL), which was significantly lower than control in the 3.4 mg/mL group at 12 h. Embryos exposed chronically (for 96 hours) to Δ^9-THC showed a significant increase in activity at 1.2 mg/mL and an habituation (i.e., no different response relative to controls) at all other doses. AM251, a cannabinoid receptor antagonist, attenuated the increased locomotor activity induced by Δ^9-THC. The authors note the results are similar to those in rodents, with dose-dependent hyperactivity followed by suppression (Akhtar et al. 2013). Morphological effects of Δ^9-THC observed include increased incidence of yolk sac edema and bent body/curved primary axis at all doses tested (0.3, 0.6, 1.2, and 2.4 mg/L), and of pericardial edema at the three highest doses tested.

In a third study employing a visual motor response test (Achenbach et al 2018), a decrease in basal activity (measured as average distance traveled) was observed in zebrafish larvae exposed to Δ^9-THC. This effect on basal activity was interpreted by the authors to be an indication of anxiogenic behavior. There were no treatment-related differences apparent at any dose in the light to dark transition activity. This study also compared the uptake kinetics and metabolism of Δ^9-THC with effects on larval behavior. The uptake kinetics for Δ^9-THC correlated with the dose-related decreases observed in the average distance traveled (Achenbach et al. 2018).

After exposure of zebrafish embryos to Δ^9-THC during a critical period of development (gastrulation) changes in heart rate, motor neuron morphology, synaptic activity at the neuro muscular junction, and locomotor responses to sound were observed (Ahmed et al. 2018). Zebrafish embryos were exposed to Δ^9-THC (0, 2, 4, 6, 8, 10 mg/L) from about 5 to 11 hpf. Significant treatment-related findings were as follows: decreased survival 5 dpf at the two highest doses; decreases in heart rate and body length at doses of 4 mg/L Δ^9-THC and above; and an increase in nicotinic acetylcholine receptors (nAChRs) and a decrease in the response to sound stimuli at 6 mg/L Δ^9-THC (Ahmed et al. 2018).

F. Epigenetic Studies in Humans and Animals

Epigenetic Effects

The potential for exposure to cannabis or Δ^9-THC to result in epigenetic effects or effects on gene or protein expression that may be the result of epigenetic changes has been investigated in human and animal studies. Table F.1 briefly describes some of the terms and concepts relevant to evaluating these studies, and Table F.2 lists the human genes evaluated in these studies, and briefly describes their functions.

Table F.1. Epigenetic terminology

DNA methylation (epigenetic modification)	Occurs when a methyl group is added at a cytosine nucleotide that precede guanines (CpG dinucleotides); influences DNA function by activating or repressing transcriptional activity of a gene and by altering chromatin accessibility and remodeling. • Frequently DNA methylation in promoter regions of genes downregulates its expression. • Higher DNA methylation in the gene body (i.e., the transcriptional region of the gene sequence, including introns and exons) may promote expression of a gene. • In most instances, DNA methylation represses gene expression by preventing the binding of transcription factors, or recruiting proteins that bind to methylated DNA (Wen et al. 2016).
Fetal epigenetic reprogramming	Resets genomic potential and erases epigenetic memory (von Meyenn and Reik 2015); occurs during the formation of primordial germ cells and in the early embryo soon after fertilization (O'Neill 2015).
Genomic imprinting (about 1% of mammalian genes)	Inherit only one working copy of these genes; depending on gene, either the maternal or paternal copy is epigenetically silenced via DNA methylation during egg or sperm formation; the epigenetic tags usually persist throughout the lifetime, but reset during egg or sperm formation; certain genes always silenced in eggs, and others in sperm(Genetics Home Reference 2019).
Histone modifications (epigenetic modification)	Histones are large groups of protein complexes that help DNA condense into chromatin. Modifications includes methylation and acetylation of lysine residues on histone tails; affect gene expression by altering chromatin structure and accessibility (Wen et al. 2016).

Table F.2 Function of genes (human) evaluated in the reviewed studies

ANKK1 Gene	Ankyrin repeat and kinase domain containing 1 (*ANKK1*). Closely linked to *DRD2* gene on chr 11; involved in signal transduction pathways
BEGAIN Gene	Brain enriched guanylate kinase associated (*BEGAIN*). Transmission across chemical synapses, protein-protein interactions at synapses; GO annotation includes *kinase activity*
CSNK1E Gene	Casein kinase 1 epsilon (*CSNK1E*). Codes a member of the casein kinase 1 protein family, whose members have been implicated in control of cytoplasmic and nuclear processes such as DNA replication and repair; central component in circadian clock
DLG4 Gene	Discs large MAGUK scaffold protein 4 (*DLG4*). Product is a protein that heteromultimerizes with another MAGUK protein and is recruited into NMDA receptor and potassium channel clusters
DLGAP2 Gene	DLG associated protein 2 (*DLGAP2*). Product is a membrane-associated protein that may play role in synapse organization and signaling in neuronal cells; biallelically expressed in brain; only paternal allele is expressed in the testis (imprinted)
DNMT1 Gene	DNA methyltransferase 1 (*DNMT1*). Major enzyme responsible for maintaining methylation patterns following DNA replication, established during development
DNMT3a Gene	DNA methyltransferase 3 alpha (*DNMT3a*). Enzyme thought to function in de novo methylation, rather than maintenance methylation and is essential for establishment of DNA methylation patterns during development
DRD1 Gene	Dopamine receptor D1 (*DRD1*). Encodes D1 subtype of the dopamine receptor; the most abundant dopamine receptor in the CNS
DRD2 Gene	Dopamine receptor D2 (*DRD2*). Encodes D2 subtype of the dopamine receptor; involved in the modulation of locomotion, reward, reinforcement and memory and learning
DRD4 Gene	Dopamine receptor D4 (*DRD4*). Encodes D4 subtype of the dopamine receptor; target for drugs that treat schizophrenia and Parkinson's disease; activated by dopamine, epinephrine, norepinephrine, and by numerous synthetic agonists and drugs; located primarily in the frontal cortex, midbrain, amygdala and the cardiovascular system
GRIN2A Gene	Glutamate ionotropic receptor NMDA type subunit 2A (*GRIN2A*). Protein coding gene for a member of the glutamate-gated ion channel protein family; GO annotations include *calcium channel activity* and *ionotropic glutamate receptor activity*
KCNA5 Gene	potassium voltage-gated channel subfamily A member 5 (*KCNA5*). Voltage-gated potassium channel subfamily member; among many cellular roles, it regulates neurotransmitter release and neuronal excitability
L1CAM (L1) Gene	L1 cell adhesion molecule (L1CAM L1). Encodes a cell adhesion protein; plays key role in multiple processes of nervous system development, including neuronal migration, axonal growth and fasciculation, and synaptogenesis

NCAM1 Gene	Neural cell adhesion molecule 1 (*NCAM1*). Encodes a cell adhesion protein; cell-to-cell interactions and cell-matric interactions during development and differentiation; involved in nervous system development and in expansion of T cells and dendritic cells (role in immune surveillance)
OPRM1 Gene	Opioid receptor mu 1 (*OPRM1*). Encodes at least three opioid receptors in humans; important role in dependence to other drugs of abuse (nicotine, cocaine, alcohol) via its modulation of the dopamine system
PENK Gene	Proenkephalin (*PENK*). Encodes a preproprotein that generates multiple *protein products, including the pentapeptide opioids, that compete with and mimic effects of opiate drugs; play role in physiological functions such as pain perception and responses to stress*
PDYN Gene	*Prodynorphin (PDYN). Encodes a preproprotein that generates multiple protein products, including the pentapeptide opioids, that compete with and mimic effects of opiate drugs; play role in physiological functions such as pain perception and responses to stress*

Epigenetic and Related Observations in Human Studies of Cannabis Use

Six studies evaluated either epigenetic endpoints or changes in mRNA gene transcript expression in cannabis exposed individuals at different life stages relevant for developmental and reproductive toxicity. Findings from these studies are briefly discussed below. Additional information on study design and study findings are presented in tabular form in Appendix 5. Many of these studies have significant limitations, including limited statistical power due to small sample size and limited exposure quantification.

Adult exposure

One study examined changes in DNA methylation in the peripheral whole blood of adults reporting cannabis use, characterizing DNA methylation status at seven specific sites within the *ANNK1*, *CBR1*, *DRD2*, and *NCAM1* genes. DNA methylation was higher in exon 8 of *DRD2*, the dopamine receptor 2 gene, and near the transcriptional start site (TSS) of *NCAM1*, the neural cell adhesion molecule 1 gene, in cannabis users compared to non-users. Increased methylation at these sites could have implications for reward conditioning and the development and maintenance of the nervous system. However, since differences in DNA methylation patterns were assessed in the somatic and not germ cells of the adult users, it is unclear if the effects would persist in the F1 generation (Gerra et al. 2018).

Another study examined changes in DNA methylation in sperm of men reporting cannabis use, compared to men reporting no use, as confirmed by urine analysis (Murphy et al., 2018). The authors reported that the methylation status of a large number of CpG sites differed between cannabis users and controls, with the majority of these differing CpG sites having lower methylation levels in the cannabis user group

(Murphy et al. 2018). Methylation of *PTG1R*, which encodes for prostaglandin 12 receptor, was reported to be significantly inversely correlated with urinary Δ^9-THC levels, while methylation of *CSNK1E*, which encodes for casein kinase 1 epsilon, was reported to be significantly positively correlated with urinary Δ^9-THC.

Prenatal exposure

Three publications from the same research group report on findings from measurements of mRNA obtained from previously collected fetal brain specimens that were characterized as to prenatal cannabis exposure based on maternal self-report and urine or fetal meconium analysis (DiNieri et al. 2011; Wang et al. 2006b; Wang et al. 2004). The study populations among the three studies appear to have a high degree of overlap; indeed, the study population for Wang et al. (2006b) and Wang et al. (2004) appears to be identical. DiNieri et al. (2011) reported a significant dose-related decrease in *DRD2* gene expression in the NAc of fetuses prenatally exposed to cannabis. As discussed in the animal studies section below, these authors also reported reduced *DRD2* mRNA in the NAc of rat pups exposed prenatally to Δ^9-THC (DiNieri et al. 2011). In a separate publication, these investigators reported decreased *DRD2* mRNA expression levels in the amygdala basal nucleus in male fetuses exposed prenatally to cannabis (Wang et al. 2004). *DRD2* dysregulation has been implicated in addiction risk and other psychiatric disorders. Alterations in levels of opioid receptor and opioid precursor mRNAs were observed in the same study population of fetuses prenatally exposed to cannabis (Wang et al. 2006b). Specifically, a significant dose-related increase in mu opioid receptor gene expression in the amygdala, a significant decrease in kappa opioid receptor gene expression in the mediodorsal thalamic nucleus, and a significant dose-related decrease in *PENK* gene expression in the caudal putamen in cannabis exposed compared to unexposed fetuses. The authors noted that disruptions in the opioid system have been implicated in the development of psychiatric disorders and adverse social and emotional behaviors (Wang et al. 2006b).

Perinatal exposure

In a nested case-control study investigating the effects of maternal cannabis use during pregnancy, buccal cell samples were collected from 8-week old infants and DNA methylation assessed at 19 specific sites in the promoter region of the dopamine receptor D4 gene *(DRD4*). Buccal epithelial cells and neuronal cells have the same developmental origin in the ectoderm, and thus buccal cells may be a useful proxy for neurodevelopmental phenotypes. Maternal cannabis use was associated with increased DNA methylation at one of the CpG sites evaluated (CpG.21.22.2). This association, which was significant after adjusting for tobacco use and other drug use, did not remain significant after application of the Bonferroni correction for multiple comparisons (Fransquet et al. 2017).

Epigenetic and Related Observations in Animal Studies of Δ^9-THC or WIN

Two studies have investigated epigenetic changes in rat offspring following preconception exposure of one or both parents to Δ^9-THC (Murphy et al. 2018; Watson et al. 2015) and one study examined epigenetic changes in rats exposed perinatally to Δ^9-THC (DiNieri et al. 2011). Three studies investigated changes in mRNA gene transcript expression, which may or may not be the result of epigenetic changes, in rat offspring following preconception exposure of one or both parents to Δ^9-THC (Szutorisz et al. 2014; Henrietta Szutorisz et al. 2016; Watson et al. 2015) one study examined changes in mRNA gene transcript expression in rats exposed prenatally to Δ^9-THC (DiNieri et al. 2011; Gómez et al. 2003), and another examined changes in mRNA gene transcript expression in rats exposed perinatally to Δ^9-THC (DiNieri et al. 2011). Also included here are rat preconception exposure studies of the CB_1R agonist WIN, including one study investigating both epigenetic and mRNA expression changes in offspring (Ibn Lahmar Andaloussi et al. 2019), and one study examining changes in mRNA expression (Ibn Lahmar Andaloussi et al. 2019; Vassoler et al. 2013). These studies differed in the timing and dose of exposure, as well as the tissues evaluated (See Appendix 5).

Preconception exposure

One study investigated epigenetic changes in sperm following exposure of sexually mature rats to Δ^9-THC (Murphy et al. 2018; Watson et al. 2015). Differentially methylated regions (DMRs) were observed within the sperm DNA of Δ^9-THC exposed rats, compared to controls (Murphy et al. 2018; Watson et al. 2015). The topmost pathways enriched with altered gene methylation patterns were identified as 'Hippo-signaling pathways' and 'Pathways in cancer'. Of interest, there were six overlapping genes in these pathways that were observed to be differentially methylated in sperm from both Δ^9-THC exposed rats, and cannabis exposed men (Murphy et al. 2018; Watson et al. 2015), suggesting that these two pathways may be targets of Δ^9-THC exposure. If DMRs are retained in the zygote, this could lead to disruption in growth regulatory genes, resulting in nonviability, or increase cancer risk later in life via fetal epigenetic programming (Murphy et al. 2018). Although the study focused on the F0 generation, some DNA methylation changes of non-imprinted genes in gametes can resist post-fertilization reprogramming and persist in the somatic cells of offspring (Tang et al. 2015). Supporting this hypothesis, the authors compared the 627 genes exhibiting DMRs in the rat sperm to the 473 DMR genes identified in the NAc of adult offspring of rats preconceptionally exposed to Δ^9-THC (compared to offspring of unexposed controls) (Watson et al. 2015). They found 55 overlapping DMR genes between these two datasets with significant enrichment, suggesting that Δ^9-THC-induced epigenetic modifications in sperm cells could persist in somatic cells. Important strengths of the

study by Murphy et al. (2018) include some validation from their findings in human sperm and from the findings in rat brain tissue of Watson et al. (2015). An important limitation is the limited statistical power.

In addition to finding numerous DMRs in the NAc of adult offspring of rats preconceptionally exposed to Δ^9-THC, Watson et al. (2015) also observed that *GRIN2A,* a component of a subset of NMDA glutamate receptors, was hypomethylated in the exposed offspring. This finding of hypomethylation of *GRIN2A* in the NAc is consistent with findings from another study by the same authors that identified reduced *GRIN2A* mRNA levels in the NAc of similarly exposed PND 32 rat offspring (Szutorisz et al. 2014). This is consistent with the hypothesis that hypomethylation in the gene bodies (i.e., the transcriptional region) may lead to decreased gene expression. Alterations in the NAc are of concern, because such changes are associated with addiction vulnerability, compulsive behaviors, and reward sensitivity.

Szutorisz et al. (2014) reported brain region-specific age-dependent changes in differential mRNA gene expression in offspring of rats preconceptionally exposed to Δ^9-THC. Specifically, significantly increased mRNA expression of *CBR1* and glutamate receptors in the NAc was observed in adolescent male rats (PND 32), while significantly decreased mRNA expression of *CBR1, DRD2*, *GRIN2A*, *GRIA1*, and *GRIA2* was observed in the dorsal striatum in adult males (PND 62) (*GRIA1* and *GRIA2* encode components of AMPA glutamate receptors) (Szutorisz et al. 2014). The same study authors further evaluated potential sex-specific effects and reported sex-specific mRNA expression patterns in both the adolescent and adult brains of offspring of rats preconceptionally exposed to Δ^9-THC (Henrietta Szutorisz et al. 2016).

Prenatal exposure

One study of the effect of prenatal Δ^9-THC exposure on gene expression was identified in rats, by (Gómez et al. 2003). The level of *L1CAM* mRNA transcripts was increased in multiple fetal brain regions of male rats exposed prenatally to Δ^9-THC (Gómez et al. 2003), which is consistent with other reports that preconceptional and prenatal Δ^9-THC exposure can alter pathways crucial for neurodevelopment.

Perinatal exposure

Histone methylation changes in the isolated NAc of fetal and adult offspring of rats exposed to Δ^9-THC during GD 5 - PND 2 were analyzed (DiNieri et al. 2011). The study reported an altered profile of specific histone methylation marks (2meH3K9 and 3meH3K4) at the *DRD2* locus in the NAc of PND 2 and PND 62 offspring of exposed rats. Decreased *DRD2* mRNA expression and decreased DRD2 binding sites in the NAc, but not the dorsal striatum, were also reported at both time points in the offspring of exposed rats, compared to controls (DiNieri et al. 2011). In contrast with the lack of effects observed on *DRD2* gene and protein expression in the dorsal striatum of adult

rats with perinatal exposure, a decrease in *DRD2* mRNA expression in the dorsal striatum was observed in adult offspring of rats exposed to Δ^9-THC prior to conception (Szutorisz et al. 2014).

Studies with the CB_1R agonist WIN

Studies evaluating alterations in DNA methylation and mRNA expression observed in rats exposed to another CB_1R agonist WIN, provide additional information that may be relevant to Δ^9-THC. One study evaluated global methylation changes in the prefrontal cortex (PFC) of adult offspring of male rats preconceptionally exposed to WIN with or without paternal exposure to unpredictable variable stress (compared to offspring of unexposed controls) (Ibn Lahmar Andaloussi et al. 2019). This study reported an increase in global DNA methylation in the PFC, with a significant interaction between stress and WIN exposure. Upregulation of mRNA of enzymes involves in DNA methylation (*DNMT1* and *DNMT3a*) was observed in the PFC of offspring of rats preconceptionally exposed to WIN. There was a correlation between global DNA methylation and *DNMT1* expression, but not between global DNA methylation and DNMT3a.

In a study of adult offspring of female rats preconceptionally exposed to WIN, an increase in *OPRM1* (opioid receptor mu 1) gene expression and no change in either *DRD1 or DRD2* gene expression was observed in the NAc in response to a morphine challenge, compared to adult offspring of unexposed females (Vassoler et al. 2013).

G. Mechanistic Considerations Related to the EC system

As noted in Section C, recognition of the importance of the EC system during early brain development has prompted considerable research in this area (Calvigioni et al. 2014; Fernandez-Ruiz et al. 2000; Fernández-Ruiz et al. 1999), however, current understanding of the mechanisms through which neurodevelopment and neurobehavior are shaped by the EC system remains limited.

As discussed in Section C, cannabinoid receptors (CB_1R, CB_2R and CB_3R) are expressed in the fetal and adult brain, however; the pattern of CBR expression in the developing brain differs from that in the mature brain. Furthermore, CBR expression within the different regions of the brain changes over the course of the prenatal and early postnatal periods, suggesting that these receptors are involved in the overall regulation of structural and functional brain maturation (Berrendero et al. 1998; Keimpema et al. 2011).

The EC system has been described as the "gatekeeper of neuronal development" (Keimpema et al. 2011). One of the main roles of the EC system is to suppress neurotransmitter release to prevent hyper-excitation of neurons by repressing excitatory postsynaptic currents (EPSCs) (Gaffuri et al. 2012; Galve-Roperh et al. 2006; Kano et al. 2009). CBR-ligand interactions within the developing brain affect synaptic plasticity, neuronal progenitor proliferation, cell differentiation, migration, axonal/neurite growth (as discussed in Section C.4), and cell fate (Duff et al. 2013; Fan et al. 2010; Kano et al. 2009; Keimpema et al. 2011), impacting neuronal structure and function, memory, cognition, and motor function. As discussed by Gerdeman and Lovenger 2003, synaptic plasticity is:

> "defined broadly as the dynamic adjustment of synaptic strength or efficacy–[it] represents a general mechanism by which environmental or internal stimuli can alter brain neuronal responsiveness, such as for the storage of information gained through experience. The durability of such changes in synaptic strength is extremely variable, such that synaptic efficacy can fluctuate with time scales ranging from milliseconds to years. It is therefore not surprising that many different cellular and molecular processes have been implicated in the plasticity of synaptic function. Long-term potentiation (LTP), a long-lasting increase in the strength of a synapse, and long-term depression (LTD), a long lasting weakening of synaptic strength, are forms of synaptic plasticity that can persist for hours to weeks…Some forms of plasticity are initiated and maintained by purely postsynaptic mechanisms, others by purely presynaptic mechanisms, and still others by mechanisms initiated in the postsynaptic neuron that are then communicated to the presynaptic neuron by the so-called retrograde messengers. These retrograde messengers are molecules released from the

> postsynaptic neuron that participate in altering the presynaptic neurotransmitter release process. Recent studies have found that in multiple forms of synaptic plasticity, postsynaptically released endocannabinoids [eCBs] function as such a retrograde signal and are critical to the alteration of synaptic efficacy" (Gerdeman and Lovinger 2003).

As indictated in the preceeding passage, the EC system regulates synaptic plasticity via retrograde signaling. Generally, this is carried out through a phenomenon called depolarization-induced suppression of inhibition (DSI) and is triggered by elevation of post synaptic Ca^{2+} concentrations, and is associated with the reduction of neurotransmitter release from presynaptic terminals (triggered by voltage dependent calcium channels [VDCCs] (Gerdeman and Lovinger 2003; Isokawa and Alger 2006). The opposite of DSI for excitatory neurotransmission is depolarization-induced suppression of excitation (DSE), which is also mediated via eCBs.

As reviewed by Kano et al. (2009), a number of different types of neurotransmitters and their receptors within the brain (e.g., N-methyl-D-aspartate (NMDA), glutamate (GLU); acetylcholine (ACh); cholecystokinin (CCK), gamma aminobutyric acid (GABA), glycine, norepinephrine (NE), dopamine (DOPA), and serotonin) are regulated by eCB signaling and CBR activation, resulting in either LTP, STD, or LTD, and thus, changes in synaptic plasticity. The molecular architecture of developing synapses is different from that of mature ones, and different components of the EC system are present in developing synapses compared to mature synapses (Maccarrone et al. 2014).

Exposures to cannabinoids can result in altered EC system signaling within the brain, affecting synaptic plasticity. Alterations in synaptic plasticity during critical periods of brain development may be of particular importance. Keimpema et al. (2011) have suggested that altered EC system signaling during development may affect brain function, leading to impaired cognitive performance, attention, and visual-motor coordination, alterations in social behavior, and increased incidence of anxiety, depression, and drug seeking behavior (Keimpema et al. 2011).

Figure 1 from (Andersen 2003) indicates the timing of several key developmental processes that occur in the human brain from conception through age 24, such as cell birth, migration, axonal and dendrite outgrowth, and synaptogenesis, and depicts periods of vulnerability to neurodevelopmental insults that can have functional consequences.

Figure 11. Stages of brain development and different windows of vulnerability**.**

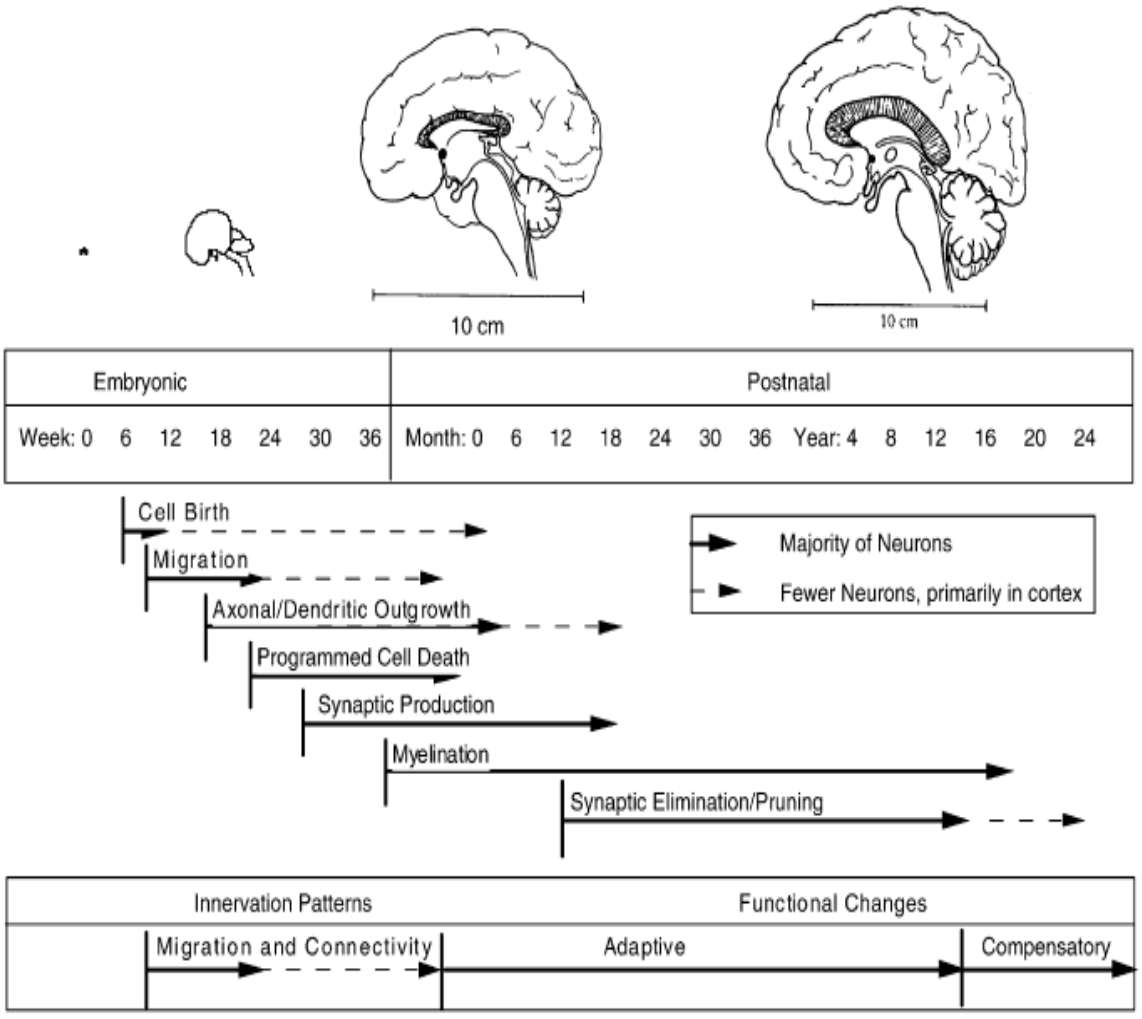


Figure 11. Taken from (Andersen 2003). “The stages of brain development (top) and different windows of vulnerability (bottom). Developmental processes occur in phases, setting the stage for potential periods of vulnerability. Insults early in life (bottom) will be assimilated into innervation patterns, whereas a later pre-pubertal insult will cause functional changes that are more adaptive.”

CB_1R Mediated Neurodevelopment Effects

Cannabinoids such as Δ^9-THC can directly activate CB_1R via the conventional GPCR mediated pathway (Alpar et al. 2016; Calvigioni et al. 2014; Maccarrone et al. 2014). Activation of CB_1R in neuronal cells can lead to a variety of different signaling cascades that can affect cellular transformation, fate decisions, inflammation (immune response), translational control, neurite outgrowth, and actin remodeling (Gaffuri et al. 2012; Keimpema et al. 2011) (Figure2). This figure has been modified to represent a very general overview of the role of the EC system in signal transduction in both inhibitory and excitatory neurons.

Figure 12. General Overview of EC System and Signaling Pathways in Excitatory and Inhibitory Neurons

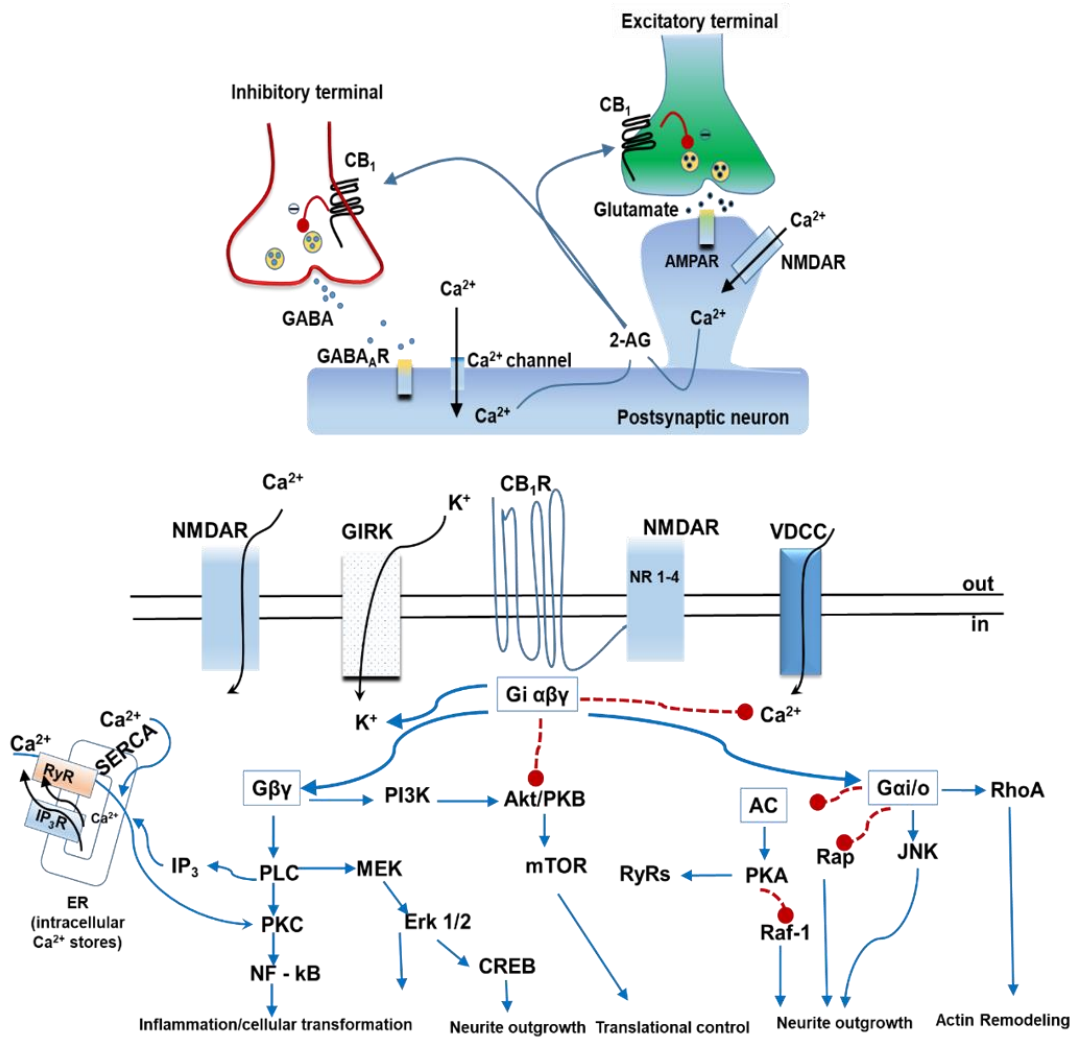


Figure 12. General overview of spatial setting of excitatory and inhibitory neurons (top) and agonist induced second messenger signaling at the CB_1R (bottom) required for proper neurodevelopment. Figure taken from (Kano et al. 2009; Keimpema et al. 2011; Sanchez-Blazquez et al. 2014; Zhuang et al. 2005) and modified. Blue Arrows: Activation; Red dotted lines: Inhibition; CB_1R can inhibit voltage-dependent Ca^{2+} channels (VDCC) or activate G-protein coupled inward rectifying potassium channels (GIRK). Abbreviations: AC, adenylyl cyclase; Akt/ PKB, protein kinase B; CREB, cAMP response element-binding protein; Erk1/2, extracellular signal regulated kinase ½; $GABA_AR$, gamma aminobutyric acid type A; GRK, G protein-coupled receptor kinase; IP3, inositol 1,4,5 triphosphate; JNK, c-Jun N-terminal kinase; MEK, Erk kinase; mTOR, mammalian target of rapamycin; Nf-κB, nuclear factor κB; p38, mitogen-activated protein kinase (MAPK); N-Methyl-D-aspartate receptor (NMDAR) PI3K, phosphoinositide-3 kinase; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; Rac, member of Rho family GTPases; Raf-1, MEK kinase; Rap-1, Ras related protein-1; RhoA, Ras homolog gene family, member A; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase pump.

Here we provide a general overview of some of the neurotransmitter (NT) systems that the EC system regulates and the potential mechanisms through which CBR agonists may act to alter the control of these systems.

CB_1R and NMDAR

Lui et al. (2009) have shown that synthetic cannabinoids such as WIN activate CB_1R and stimulate the release of Ca^{2+} from intracellular stores in dorsal root ganglion (DRG) neurons (Figure 12). This raises cytosolic Ca^{2+} levels (via the inositol 1,4,5-triphosphate receptor [IP3R] pathway) and inhibits NMDA-mediated Ca^{2+} influx (Liu et al. 2009).

The CB_1R is physically associated with the NMDAR (Sanchez-Blazquez et al. 2014). The NMDAR is one of three types of glutamate receptors, the other two being α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainic acid (kainate) receptors. NMDARs are involved in normal synaptic transmission and their activation is associated with induction of synaptic plasticity in the CNS, such as LTP and long term depression (LTD) in the hippocampus. Involvement of AMPA and kainate receptors is also necessary for the depolarization of the post synaptic membrane to facilitate LTP (Bloom 2006b).

Cannabinoid-mediated control of NMDARs modulates excitotoxicity in neurons by negative regulation. The C-terminus of the CB_1R can be directly linked to the N1 subunit of the NMDAR through the scaffolding protein HINT1 (histidine triad nucleotide-binding protein 1). Through this close association with NMDARs, CB_1Rs are able to negatively regulate excitatory neurotransmission. Over-activation of CB_1Rs by ligands can cause glutamatergic hypo-function, which can lead to dopaminergic hyper-function in the limbic areas and the cortex (Sanchez-Blazquez et al. 2014).

After prenatal exposure of rats to the synthetic CB_1R agonist WIN hippocampal gluatamate levels were decreased, and memory deficits linked to dysfunction in hippocampal LTP and glutamate release (i.e., glutamatergic hypofunction) were observed (Mereu et al. 2003). Three additional studies of rats prenatally exposed to CB_1R agonists (WIN, Δ^9-THC) have also reported neurochemical and behavioral effects consistent with glutamatergic hypofunction and impaired memory (Antonelli et al. 2004; Antonelli et al. 2005; Castaldo et al. 2007; Mereu et al. 2003). Specific effects observed include a decrease in basal glutamate levels (Antonelli et al. 2004; Antonelli et al. 2005), an increase in GLT1 (glutamate transporter) expression in frontal cerebral cortex (Castaldo et al. 2007), impairment in cortical glutamatergic neurotransmission, abnormal neurite outgrowth (Antonelli et al. 2005), and impairment of memory retention associated with alterations of hippocampal LTP and glutamate outflow (Antonelli et al. 2005). Taken together, these studies suggest that prenatal exposure to CB_1R agonists have the potential to induce glutamatergic hypofunction, likely through inhibition of NMDAR function, and thereby affect synaptic plasticity of the developing brain, and impact neurological functions, such as memory.

Antagonism of NMDARs within the hippocampus during synaptogenesis has been proposed, through a series of molecular and cellular events, to result in the impairment of learning and memory, and this series of events is presented schematically as an adverse outcome pathway (AOP) on the Organization for Economic Co-operation and Development's (OECD) AOP webpage (https://aopwiki.org/aops/13) (Figure 13). The AOP concept, depicted in Figure 14, can be useful in considering hypotheses about mechanistic data and how they relate to adverse outcomes.

Figure 13. Impaired Learning and Memory AOP for NMDAR Antagonists (https://aopwiki.org/aops/13)

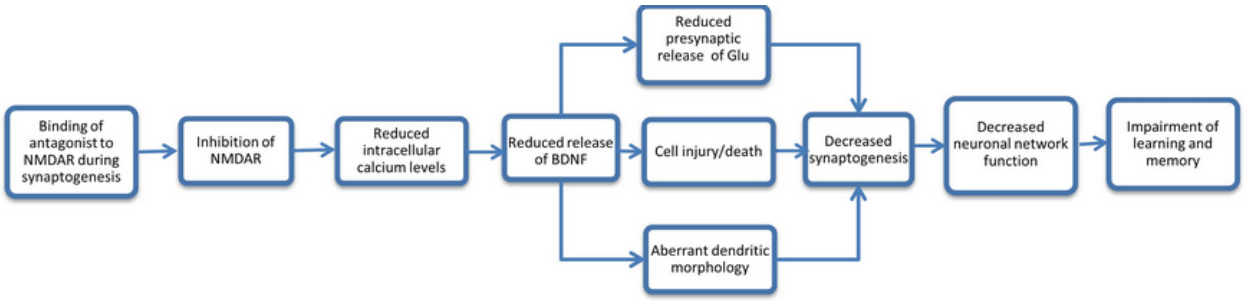


Figure 14. General Schematic of an AOP

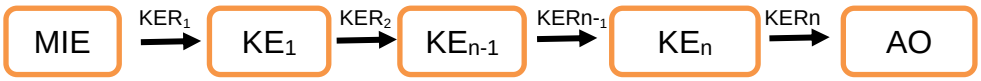
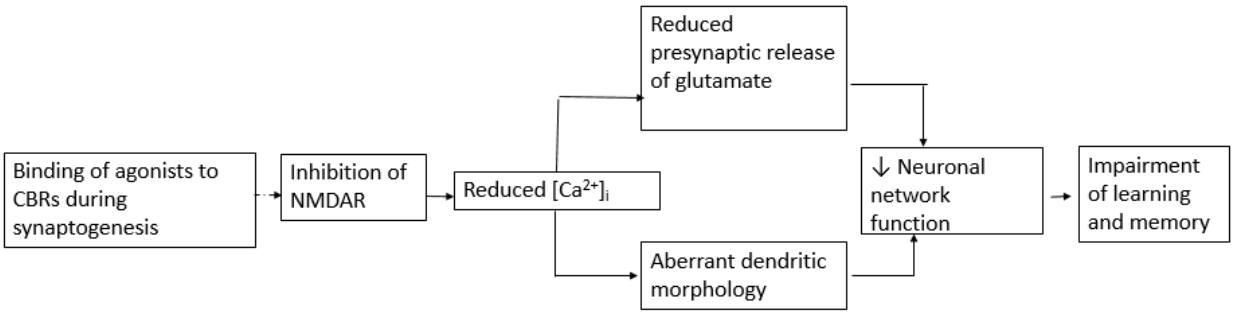


Figure 14. Adverse outcome pathways (AOPs) focus on describing critical steps along the path to an adverse outcome (AO), which can be measured (The Organisation for Economic Co-operation and Development 2017). The steps in the AOP begin with a stressor that interacts with a biomolecule in an organism such as a receptor that causes a disturbance in the normal sequence of events. This is called a molecular initiating event (MIE). The MIE progresses through a dependent series of key events (KEs) that lead to an adverse outcome (AO).

Given the findings from the studies of (Antonelli et al. 2004; Antonelli et al. 2005; Castaldo et al. 2007; Mereu et al. 2003) described above, we have modified the AOP shown in Figure 3, to incorporate CBR agonism and the first step (i.e., the molecular initiating event) in the pathway (See Figure 5). In this modified and hypothetical AOP, the binding of agonists to CBRs during synaptogenesis is postulated to inhibit NMDARs, which then triggers other key events (KE) that ultimately lead to impairment of learning and memory.

Figure 15. *Hypothesized* Impaired Learning and Memory AOP for CBR Agonists



CB_1R and GABA Mediated Δ^9-THC Effects

In addition to controlling excitatory neurotransmission, CB_1R has a role in modulating inhibitory neurotransmission by controlling the release of GABA, more commonly known as retrograde suppression of inhibition (Bloomfield et al. 2016). GABAergic synapses undergo morphological and physiological plasticity during neurodevelopment (Wang and Armstrong 2012) and the GABAergic system has been shown to influence neuronal cell proliferation, migration, and synapse formation during embryonic development and during the first week after birth (Beggiato et al. 2017; Berghuis et al. 2007). Perturbation of the GABAergic system by exogenous cannabinoids is reported to cause permanent changes in neuronal signaling, brain architecture, information processing, and behavior (Beggiato et al. 2017). Perinatal exposure to Δ^9-THC was observed to reduce GABA uptake and outflow and CB_1R density in hippocampal neurons, and reported to impair GABAergic neurotransmission in adulthood (Beggiato et al. 2017). Investigators have reported that prenatal exposure to the CB_1R agonist WIN alters the migration of GABAergic interneurons in the rat cerebral cortex (Saez et al. 2014).

Δ^9-THC and Dopamine

The EC system also has a role in controlling dopaminergic neurons. Endocannabinoids are involved in dopaminergic pathways and modulate retrograde feedback on presynaptic glutamatergic and GABAergic nerve terminals which control dopamine transmission. Gestational Δ^9-THC exposure has been associated with increased dopamine receptor and tyrosine hydroxylase (a key enzyme involved in dopamine synthesis) gene expression in the fetal brain of rats (Bloomfield et al. 2016). In rats that were prenatally exposed to Δ^9-THC there was an observed reduction in the eCB-induced firing of dopaminergic neurons. Δ^9-THC is also reported to affect the morphology of dopaminergic neurons. When significant, structural abnormalities can affect neuronal network wiring, potentially leading to adverse neurobehavioral outcomes (Bloomfield et al. 2016).

CB_1R and Ca^{2+}

As shown in Figure 2, activation of CB_1R evokes transient Ca^{2+} elevation via phospholipase C (PLC) and other VDCCs. Calcium homeostasis's role in synaptic plasticity and proper neuronal development in terms of excitatory and inhibitory neurons, neurotransmitters, and their receptors is crucial to proper neuronal development and function; however Δ^9-THC appears to affect other targets such as various ion channels and changes Ca^{2+} homeostasis. In zebrafish embryos, exposure to Δ^9-THC during the critical period of development (gastrulation) was reported to change heart rate, motor neuron morphology, synaptic activity at the NMJ, and locomotor responses to sound (Ahmed et al. 2018), all of which could relate to changes in Ca^{2+} homeostasis during neurodevelopment.

CB_1R activation activates A-type Ca^{2+} channels and GIRK (mentioned below); it inhibits N/P/Q-type Ca^{2+} channels and D and M-type K^+ channels (Fisyunov et al. 2006; Kano et al. 2009). It has also been shown that the CB_1R agonist WIN inhibits L-type Ca^{2+} channel current in GT1-7 hypothalamic neurons (Hoddah et al. 2009; Yang et al. 2016). Furthermore, Yang et al. 2016 showed that the L-type Ca^{2+} channels (LTCC) current was suppressed by WIN in a CB_1R-independent manner. LTCCs are present in various tissue types including the CNS and skeletal muscle. In the skeletal muscle, they are coupled to ryanodine receptors (RyRs) and play a pivotal role in the process of excitation contraction coupling (ECC) (Pessah et al. 2010). RyRs are expressed in the CNS (pre and post synaptically) and PNS. In the CNS, RyRs play a pivitol role in modulating neurochemical and structural aspects of synaptic plasticity (Pessah et al. 2010).

ECC is the process by which depolarization of the plasma membrane activates LTCCs (the dihydropyridine receptors-DHPR), which are coupled to RyRs to release Ca^{2+} from the sarcoplasmic reticulum that leads to muscle contraction (Olah et al. 2016). ECC involves a very tight interaction between RyRs and LTCCs; disrupting this coupling can have developmental effects later on (Kaplan et al. 2018). Prenatal exposure to Δ^9-THC has been observed to change motor behavior in some animal studies. The EC system has been found to play a pivotal role in myoblast to myotube differentiation where 2-AG acts as a repressor of myoblast differentiation via CB_1R-mediated inhibition of Kv7.4 channels (Maccarrone et al. 2015b) and have a constituitively active role in negatively regulating ECC. LTCC-mediated Ca^{2+} signaling is crucial for proper patterning of and synapse formation at the neuromuscular junction (NMJ) and dysfunctional Ca^{2+} signaling causes aberrant nerve branching that can have an effect on motor function. Others such as Betzenhauser and Marks 2010 concluded that

"CB_1R-mediated signalling in skeletal muscle inhibits the RyR-mediated Ca^{2+} transients via a Gi/o protein and PKA-dependent way, alters the activity of SERCA, reduces the Ca^{2+}-sensitivity of the contractile proteins and increases muscle fatigue".

Therefore, it can be hypothesized that exocannabinoids can cause motor dysfunction via CNS effects (altered neuronal function and plasticity), PNS effects such as aberrant branching at the NMJ, and/or developmental issues with the skeletal muscle itself.

CB_1R and GIRK

It has been shown that CB_1R and GIRK channels are co-localized in many of the areas of the CNS and play an inhibitory role in neurotransmitter release (McAllister et al. 1999). Agonist binding to CB_1Rs leads to activation of GIRK channels which result in a more negative membrane potential (hyper-polarization), thus decreasing spontaneous action potentials and ultimately inhibiting the release of excitatory NTs (Andersen et al. 2018).

TRP Receptors: The "Ionotropic Cannabinoid Receptors"

The transient receptor potential (TRP) family of receptors are nonselective cation channels that are not characterized as cannabinoid receptors because they are not strongly activated by 2-AG; however, they can be activated by AEA (Kano et al. 2009). The mammalian TRP superfamily consists of six subfamilies: canonical (TRPC), vanilloid (TRPV), polycystin (TRPP), mucolipin (TRPML), ankyrin (TRPA), and melastatin (TRPM). Some of these receptors have also been reported to be activated by Δ^9-THC. Specifically, Δ^9-THC was reported to act most potently with TRPV2, and to moderately modulate TRPV3, TRPV4, TRPA1, and TRPM8, but not TRPV1 (Muller et al. 2019).

TRPV2 is widely expressed in sensory neurons and is thought to play a role in neurodevelopment, smooth and striated muscle function, immune function, and endocrine function. During neurodevelopment, TRPV2 is expressed in mouse DRG and spinal motor neurons from embryonic day 10.5 until embryonic day 13.5. TRPV2 has been shown to be involved in regulating axon outgrowth through a Ca^{2+} -mediated mechanism. Interestingly, TRPV2 knockout mice have reduced perinatal viability (Peralvarez-Marin et al. 2013).

Epigenetic Effects of Δ^9-THC

It has been suggested that maternal cannabis use can alter epigenetic regulatory mechanisms, leading to persistent abnormal gene expression levels which may contribute to the neurobehavioral effects reported in the offspring (Morris et al. 2011). As reviewed previously, the eCB system plays a crucial role in neurodevelopment

DNA methylation alterations of the nucleus accumbens (NAc) of adult progeny was associated with their prenatal exposure to Δ^9-THC prenatally (Melis et al. 2017). Differentially methylated regions (DMRs) within loci that include genes that are involved in behavioral and physiological trait characteristics were observed to be significantly enhanced after prenatal exposure to Δ^9-THC. Cross-generational epigenetic alterations

in the NAc associated with Δ^9-THC exposure, includes DMRs localized to genes that regulate synaptic plasticity and glutamatergic transmission such as glutamate and kainite receptors, GPCRs, pre- and postsynaptic ion channels, and scaffolding proteins (Melis et al. 2017; Watson et al. 2015). Watson et al. (2015) reported that parental Δ^9-THC exposure is associated with epigenetic perturbations involving DNA methylation in the subsequent generation. Therefore, Δ^9-THC exposure before mating may be associated with molecular changes that may continue through cross generational DNA methylation in the NAc of unexposed adult progeny. Changes in striatal glutamate receptor subunits, measured as changes in mRNA and protein levels, were observed in adult F1 progeny from parents exposed to Δ^9-THC (H. Szutorisz et al. 2016). These changes were associated with dysregulation of synaptic plasticity as measured by electrophysiology studies (Watson et al. 2015).

There have been several processes described that elucidate the EC system's modulatory role in the regulation of specific processes during neurodevelopment. These processes include: regulation of gene expression of key proteins for neurotransmitters (e.g. tyrosine hydroxylase and opiod precursor proenkephalin); regulation of apoptotic death of specific neurons (Bcl-2/Bax); and modulation of gene expression and/or function of neural adhesion molecules (neural adhesion molecule L1) (Fernandez-Ruiz et al. 2004).

Apoptosis is a routine process in the adult stage; however, it plays an imperative role during neurodevelopment where it acts to terminate neurons that do not reach their targets or are in excess. Apoptosis is executed via a cascade or proteases (caspases) and in the process of neurodevelopment the Bcl-2/Bax system is a mediator of caspases that controls apoptosis. Endocannabinoids promote apoptosis via CB_1R/CB_2R and have been found in neurotrophic neurons. Exocannabinoids could potentially alter the normal caspases leading to programmed cell death in neurons which can have detrimental effects in proper neuronal functioning (Fernandez-Ruiz et al. 2004).

In the case of N-CAM (L1), a key protein in neural development, Δ^9-THC is associated with an increase in the expression of this gene. The N-CAM protein is found in neurons and glial cells and is involved in cell-to-cell and cell-to-matrix interactions during neurodevelopment such as cell proliferation and migration, neuritic elongation and guidance, synaptogenesis and myelinogenesis. Disruption of this gene in mice has been reported to cause brain abnormalities (Fernandez-Ruiz et al. 2004).

Appendix 1. Human Developmental Studies of Cannabis and Δ^9-THC: Birth Outcomes

Appendix Tables 1.1. Human Studies: Birth Outcomes

Study/ Location	***Study Design, Sample Sizes, Statistical Analysis***	***Outcomes of Interest***	***Exposure Measurement Methods***	***Exposure quantification***	***Results***	***Covariates/ Confounders***	***Comments***
Bada et al. 2005 RI, FL, TN, MI Maternal Lifestyle Study	Retrospective cohort 1993 - 1995 (Lester et al. 2001) N = 19,079 Mother-infant dyads were recruited shortly after delivery if singleton birth, BW ≥500g, and GA <43 wks Recruitment targeted all VLBW infants (Lester et al., 2001) 16,988 dyads met eligibility criteria 11,811 consented to participate; 3,174 participants excluded for insufficient meconium for analysis n = 8,637 Mothers were interviewed about substance use and prenatal care. Data were abstracted from maternal and infant medical records Chi-squared test, *t*-test, logistic regression	LBW IUGR PTB	Self-report from interview at birth	Cnb was reported as a binary variable. Participants who reporting use during preg were considered +ve for Cnb Cocaine and opiate exposure were based on self-report or meconium analysis	LBW: OR = 1.21 (0.9, 1.61) IUGR: OR = 1.08 (0.85, 1.36) PTB: OR = 0.9 (0.73, 1.11)	Included: Clinical site Legal and illegal drug use Tobacco Alcohol Interaction term for cocaine and GA (LBW and IUGR models) Considered: Maternal medical and obstetric complications Any hospitalization during preg Maternal weight gain during preg Prenatal care Maternal age Medicaid insurance Infant's gender Race	Strengths: Large sample from multiple locations Limitations: Large percentage of eligible dyads did not participate Prevalence of Cnb use was 9.4% among the included dyads and 3.9% among the dyads excluded for insufficient meconium, p<0.001 Details about Cnb exposure assessment, e.g., screening questions, exposure periods, frequency, quantity, not reported

Study/ Location	***Study Design, Sample Sizes, Statistical Analysis***	***Outcomes of Interest***	***Exposure Measurement Methods***	***Exposure quantification***	***Results***	***Covariates/ Confounders***	***Comments***
Chabarria et al. 2016 Houston, TX	Retrospective cohort 2011 - 2015 Identified participants through a perinatal database Included all women who delivered singletons at an affiliated hospital N = 13,919 Exclusions: delivered at nonaffiliated hospital, missing information about smoking during preg, insufficient delivery or postnatal information n = 12,069 singleton births Participants were interviewed about use of Cnb and tobacco upon presentation to labor and delivery Mann-Whitney *U*-test, Chi-squared test, logistic regression	PTB (22-37 wks) Early PTB (22-34 wks) HC<25th percentile HC<2 SD below mean FGR (<10th percentile) 5-minute Apgar score<7 BW<25th percentile Preterm premature rupture of membranes (PPROM) Placental abruption	Interview w/ open-ended and directed questions to determine current and ever use of Cnb, and quantity Cnb use was defined by self-reported use during preg	Cnb and tobacco use during preg were analyzed as binary variables Cnb only (n=58, 0.48%) Tobacco only (n=194, 1.61%) Tobacco and Cnb dual use (n=48 0.4%) Nonsmokers (n=11,769, 97.5%)	ORs (CI) for Cnb alone, tobacco alone, and dual use of Cnb and tobacco: PTB Cnb 0.84 (0.35, 3.87) Tobacco 1.63 (1.12, 2.38) Dual use 2.56 (1.33, 4.94) HC <25th percentile Cnb 1.44 (0.82, 2.53) Tobacco 1.67 (1.20, 2.33) Dual use 2.34 (1.27, 4.31) BW <25th percentile Cnb 1.09 (0.61, 1.95) Tobacco 2.09 (1.55, 2.83) Dual use 2.79 (1.55, 5.04) HC<2 SD below mean, FGR, Early PTB, 5-minute Apgar<7, PPROM, placental abruption were not assoc w/ Cnb alone or dual use	Included Cigarettes Age Parity Race/ethnicity Marital status Chronic hypertension Diabetes (pregestational and gestational)	Very low prevalence of Cnb use. Authors attribute this to lower rates of Cnb use among Hispanic populations.

Study/ Location	*Study Design, Sample Sizes, Statistical Analysis*	*Outcomes of Interest*	*Exposure Measurement Methods*	*Exposure quantification*	*Results*	*Covariates/ Confounders*	*Comments*
Coleman-Cowger et al. 2018 Baltimore, MD	Prospective cohort 2017 - 2018 N = 1,170 women approached in 2 obstetric clinics as part of a study screening for illicit and prescription drug use during preg. Of 719 women who met eligibility criteria (≥18 yo, English proficiency to provide consent, and natural hair length ≥3 cm for drug testing), 500 were enrolled n = 338 live, singleton births (excluding SB/SAB) Logistic regression, ANOVA, chi-square, propensity score weighted regression for outcomes w/ <8 outcomes per covariate	LBW BL HC PTB SB/SAB BD NICU admission Apgar scores Obtained from electronic medical records (EMR) Cutoffs for girls and boys, respectively, were: LBW <2400, <2500 g Low BL <45.4, <46.1 cm Low HC <31.5, <31.9 cm	Interview-administered demographic and substance use screening using 4P's Plus questionnaire regarding substance use in mo prior to knowing preg EMR chart review, for previously documented histories of substance use to confirm survey responses Drug screen results to validate survey responses, using urine for short-term substance use detection and hair samples for use up to 90 days Authors assessed frequency of Cnb use, but not used in final analysis	Days of past-mo Cnb use (total n = 500): Did not use, n=393 (78.9%) <1 day/wk, n=17 (3.4%) 1-2 days/wk, n=23 (4.6%) 3-6 days/wk, n=24 (4.8%) Every day, n=41 (8.2%) Cnb and tobacco use categories (total n=338): Cnb only, n=36 Co-use of Cnb and tobacco, n=22 Tobacco only, n=23 No use, n=257	All ORs: no use is referent <u>LBW</u> Cnb-only: 1.0 (0.1, 7.9) Co-use: 0.7 (0.1, 8.6) <u>Low BL</u> Cnb-only: 2.0 (0.3, 11.9) Co-use: 1.4 (0.1, 14.2) <u>Low HC</u> Cnb-only: 2.0 (0.4, 10.6) Co-use: **5.7 (1.1, 28.9)** <u>PTB</u> Cnb-only: 2.2 (0.8, 5.6) Co-use: 1.7 (0.5, 5.8) <u>SB/SAB</u> Cnb-only: **12.1 (1.03, 141.8)** Co-use: 10.1 (0.8, 130.7) <u>BD</u> Cnb-only: 1.2 (0.5, 2.9) Co-use: **3.1 (1.2, 8.3)** <u>NICU admission</u> Cnb-only: 1.5 (0.6, 3.6) Co-use: 1.9 (0.7, 5.7) Tobacco-only use was ns assoc w/ birth outcomes	Included in analyses indicated below: Alcohol[5] Other drug use (opiates, stimulants & benzo-diazepines)[5] Race/ethnicity[3,5] GA[1,2,3] Gravidity[4] Education level[8] Marital status[2,6] Trimester of enrollment[2,6] Preg intention[7] Maternal age[4] [1] LBW model [2] Low BL model [3] Low HC model [4] PTB model [5] SB/SAB model [6] BD model [7] NICU model [8] Low Apgar score	Prevalence of Cnb-only use is higher than prevalence of tobacco-only use. BD were only defined as the "presence or absence of birth defects (e.g., cardiac; musculoskeletal; gastrointestinal)". <u>Strengths</u>: Validated survey results w/ drug screen tests from urine and hair samples. <u>Limitations</u>: Authors used survey responses, "validated w/ EMR data and drug testing," but do not explain how they handled discrepancies in exposure based on the different data sources.

Study/ Location	***Study Design, Sample Sizes, Statistical Analysis***	***Outcomes of Interest***	***Exposure Measurement Methods***	***Exposure quantification***	***Results***	***Covariates/ Confounders***	***Comments***
Conner et al. 2016 US, Australia, the Netherlands, UK, Canada, Jamaica, Spain, France, New Zealand	Meta-analysis 2,693 studies were identified, after exclusion criteria were applied, 31 studies were included in meta-analysis 13 prospective cohort, 13 retrospective cohort, 5 case-control Studies were excluded if they included Cnb users in the control group, included a duplicated cohort, outcome data for Cnb users could not be extracted from other drug use, or if raw data could not be extracted Case series, case reports, abstracts, unpublished data, expert opinions, review articles, animal studies, and non-English publications were also excluded Meta-analysis identified the quality of studies based on the following factors: Objective definition of Cnb use Quantified Cnb use Excluded other substance use Adjusted for tobacco Identified risk for selection bias	BW LBW SGA GA PTB SB SAB Perinatal death NICU admission Placental abruption Apgar (“Primary outcomes” were LBW and PTB)	Each study used self-report alone (19), biological measures alone (4), or a combination of the two (7) Biological measures included urine (5), meconium (5), umbilical cord (2), serum (2) and oral fluid (1)	Exposure was classified as Cnb use if participant reported any Cnb during preg Cnb use was stratified by amount: Low (less than weekly) Moderate (“weekly or stated moderate”) High (“daily or stated heavy”) Unexposed group Women who did not use Cnb during preg Exposure was further stratified by co-use of tobacco use, where possible	<u>LBW</u> *Pooled Estimates* Overall (12 studies): RR = **1.43 (1.27, 1.62)** Data stratified by frequency of Cnb use (2 studies): Low use: RR = 1.22 (0.91, 1.64) Moderate use: RR = **1.90 (1.44, 2.45)** *Pooled adjusted estimates (adjusting for confounders including tobacco use)* (4 studies) LBW: OR = 1.16 (0.98, 1.37) <u>PTB</u> *Pooled Estimates* Overall (14 studies) RR = **1.32 (1.14, 1.54)** Data stratified by frequency of Cnb use: (5 studies for low and moderate use, 2 studies for high use) Low use: RR = 1.09 (0.91, 1.32) Moderate use: RR = **2.04 (1.32, 3.17)** High use: RR = **1.73 (1.09, 2.73)** Stratified by tobacco use (2 studies): Cnb only (compared w/ no	Estimates for most outcomes were stratified by concurrent tobacco use Socioeconomic and demographic factors that were assessed as potential confounders varied across studies For several outcomes, estimates from studies that adjusted for various socioeconomic and demographic factors were separated from unadjusted estimates	Results observed that maternal Cnb use during preg is not an independent risk factor for LBW or PTB after adjusting for factors such as tobacco use (4 studies for each outcome). There also does not appear to be an increased risk for other adverse outcomes such as SGA and placental abruption after accounting for other influencing factors. Authors stated “these data suggest that the association between maternal marijuana use and adverse pregnancy outcomes may be attributable to concomitant tobacco use and other confounding factors and not marijuana alone.” Authors observed that “women who used marijuana during pregnancy were at increased risk for lower mean birth weight, stillbirth, and low Apgar score based on pooled unadjusted estimates, but adjusted estimates were not able to be pooled for these secondary outcomes”.

Study/ Location	Study Design, Sample Sizes, Statistical Analysis	Outcomes of Interest	Exposure Measurement Methods	Exposure quantification	Results	Covariates/ Confounders	Comments
	Excluded multiple gestations or fetal anomalies Studies that met 3 of the 6 criteria were included n = 7,851 Cnb users n = 124,867 non-users DerSimonian-Laird Random-effects model, pooled RR, standard mean difference (MD) pooled OR, Cochran's Q and Higgin's I^2 test for heterogeneity, Harbord test, Egger test				Cnb, no tobacco): RR = 1.25 (0.63, 2.50) Cnb and tobacco use (compared w/ no Cnb, no tobacco): RR = **1.85 (1.21, 2.81)** *Pooled adjusted estimates (adjusting for confounders)* (4 studies) OR = 1.08 (0.82, 1.43) *Outcomes based on pooled, unadjusted estimates* BW (8 studies): MD = **-167g (-245, -90)** SB (2 studies): OR = **1.74 (1.03, 2.93)** SAB (2 studies): OR = 1.10 (0.84, 1.44) NICU admission (5 studies): RR = 1.41 (0.99, 2.0) Apgar score (6 studies): RR = **1.26 (1.07, 1.40)** GA (6 studies): MD = -0.1 wks (-0.5, 0.3) Including only data from studies that adjusted for tobacco, some of which also adjusted for other drug use and/or other demographic factors: SGA (4 studies): aOR = 1.59 (0.87, 2.31)		Strengths: Analyses included stratification based on frequency of use, and on concurrent tobacco use (however, this resulted in the inclusion of a much smaller no. of studies). Adjusted and unadjusted risk estimates were provided. Each study was graded on its quality based on a defined grading scale of 6 factors. The influence of potential confounders was examined when possible. Heterogeneity was determined using both Cochran's *P* and Higgins's I^2 tests, and stratified analysis was conducted based on the results (however, this resulted in the inclusion of a much smaller no. of studies). Limitations: Varying assessment methods by each study may dilute or distort findings. Cnb users who also used other substances were included in the exposed group.

Study/ Location	Study Design, Sample Sizes, Statistical Analysis	Outcomes of Interest	Exposure Measurement Methods	Exposure quantification	Results	Covariates/ Confounders	Comments
					Placental abruption (2 studies): aOR = 1.35 (0.28, 2.42)		None of the LBW studies stratified by tobacco use. Two studies of PTB stratified by tobacco use; however, the resulting no. of women in the analysis who used Cnb during preg was very small. There was considerable heterogeneity (based on Cochran's *P* and I^2) among studies for the following outcomes in the pooled estimates w/out adjustment –LBW, PTB, BW, GA, SGA, NICU. Authors state that for the secondary outcomes that included case–control studies, pooled ORs were used. However, outcomes for pooled adjusted estimates were reported as ORs for LBW and PTB while none of the included studies were case-control studies.

Study/ Location	*Study Design, Sample Sizes, Statistical Analysis*	*Outcomes of Interest*	*Exposure Measurement Methods*	*Exposure quantification*	*Results*	*Covariates/ Confounders*	*Comments*
Cornelius et al. 1995 Pittsburgh, PA	Prospective cohort Participants recruited from a prenatal clinic at Magee Womens hospital 1990 - 1994 <u>Inclusion criteria:</u> ≤18 yrs ≤5 mos gestation N = 329 10 participants moved, 4 had spontaneous abortions at <20 wks gestation, there were 2 sets of twins, and 2 stillborn infants n = 310 Stepwise linear regression model, logistic regression, Wald's *t* test Cnb use per trimester was dichotomized due to small sample in 2nd and 3rd trimester	BW LBW BL HC PI SGA GA BD Chest circum-ference Apgar Scores	Self-report by interview performed during 1st trimester and another w/in 36 hours of delivery The first interview contained questions about Cnb, alcohol, and tobacco use during the yr prior to preg and in the 1st trimester The 2nd interview collected the same information for the 2nd and 3rd trimesters	Cnb use was expressed as average daily joints (ADJ), and categorized: Light: 0-0.4 joints/day Moderate: 0.41-0.88 joints/day Heavy: ≥0.89 joints/day Cnb use was analyzed per trimester Hashish and sinsemilla were converted to joints in a 3:1 and 2:1 ratio, respectively. Blunts were converted to 4 joints (Only 1 teenager used hashish before preg and in the 1st trimester. No one reported using sinsemilla)	Average maternal age=16 yrs (range 12-18) Cnb use: 1st trimester (17%) 2nd trimester (5%) 3rd trimester (3%) First trimester Cnb "use (any) was significantly assoc w/ a decrease in GA of 7 days" when controlling for covariates (significance level not presented) ADJ was not assoc w/ any growth or morphological outcomes (statistics were not presented) Second trimester Cnb use was assoc w/ increased risk of SGA: OR = **3.8 (1.2, 14)** Among white individuals 1st trimester Cnb use was assoc w/ increased risk of minor physical anomalies: OR = 3.2 (1.0, 10.2)	<u>Included:</u> GA Race Infant sex Maternal age Maternal height Gravidity Pre-preg weight Adequacy of prenatal care Maternal nutrition Household structure Social support Depression Teenager's parent's education School status Alcohol use Cocaine/crack Other drugs In the regression analysis alcohol, tobacco and other drug use were controlled for in the corresponding time period	70% of the women were African-American and of predominantly low SES Since only 15 and 9 teenagers used Cnb in the 2nd and 3rd trimesters respectively, use was dichotomized as user/non-user in the regression analysis A significant decrease in GA was observed despite a small no. of teenagers using Cnb in the 1st trimester <u>Strengths:</u> Quantified Cnb and tobacco use, and analyzed per trimester Accounted for various methods of Cnb use Assessed Cnb use in the yr before preg <u>Limitations:</u> Small sample of Cnb users, particularly in the 2nd and 3rd trimesters

Study/ Location	***Study Design, Sample Sizes, Statistical Analysis***	***Outcomes of Interest***	***Exposure Measurement Methods***	***Exposure quantification***	***Results***	***Covariates/ Confounders***	***Comments***
Cornelius et al. 2002 Pittsburgh, PA MHPCD	Prospective cohort Participants recruited from a prenatal clinic at Magee Womens hospital Births: 1990 - 1994 Follow-up: 1996 - 2000 <u>Eligibility criteria:</u> ≥4 mo gestation ≤18 yo N = 448 adolescents (avg age 16 yrs) were approached 445 enrolled and interviewed Exclusions: moved away, refused delivery interview, multiple births, SAB, still birth n = 413 interviewed at delivery n = 345 mother-infant dyads evaluated at 6 yrs Multiple regression	Mother-infant dyads were examined at birth, and again at 6 yrs postpartum Growth outcomes at 6 yrs: Weight Height Skinfold thickness HC BMI PI Weight-for-height Z scores	Three in-person interviews at 4 or 5 mos gestation, during postpartum hospital stay, and at 6 yrs assessed drug use for the yr before preg and 1st trimester, 2nd and 3rd trimesters, and the 6 postpartum yrs, respectively	Cnb assessed as average no. of Cnb joints smoked/day during preg Hashish bowls, blunts, and sinsemilla joints were counted as 3, 4, and 2 Cnb joints, respectively 1st trimester Cnb use was analyzed as a continuous variable, 2nd and 3rd trimester use were recorded as binary variables due to sample size	Any Cnb exposure during the 2nd trimester predicted a **decrease of 1.1 inches (p<0.01)** in <u>height</u> at 6 yrs No other assoc btwn growth outcomes at 6 yrs and prenatal Cnb exposure were reported	<u>Included</u>: Work Ethnicity Maternal coping Religious service attendance Home environment Maternal custody of child Infant gender Infant age Hospitalizations Child appetite Maternal height Paternal height Gravidity Prenatal use of alcohol and/or tobacco <u>Considered:</u> Maternal education Maternal age Family income Male in household Any other adult in household Maternal depression Maternal hostility Life events Social support No. of illnesses No. of injuries No. of children in house Nutrition	Sample was 69% African-American and 31% Caucasian, predominantly lower SES Prenatal substance exposure and demographics were similar for those who participated through the 6 yr evaluation and those who left the study <u>Strengths:</u> Measured substance use by trimester and accounted for quantity Questions and measures to assess substance use were developed and tested for studies of alcohol use during preg in adult women Accounted for variation of Cnb use method Controlled for a wide range of variables, including nutrition, appetite, home environment, social support, environmental tobacco smoke <u>Limitations:</u> Self-report exposure assessment Small sample of 2nd and 3rd trimester Cnb users

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						Environmental tobacco exposure Current maternal Cnb, tobacco, or alcohol use	

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Day et al. 1991 Pittsburgh, PA MHPCD	Prospective cohort Participants recruited from prenatal clinic at Magee Womens hospital 1982 - 1985 Eligibility criteria: >18 yo >4 mos preg N = 1,360 women interviewed at 4 mo prenatal visit Women were included in the study if they reported ≥2 joints Cnb/mo during the 1st trimester w/ the same no. of controls Women were excluded for the analysis for each trimester where they did not perform interview 10 women lost to follow up, 2 fetal deaths, and 22 additional exclusions for absence of delivery interview n = 519 Multiple and logistic regression, ANCOVA, chi-square	BW BL PI SGA GA PTB BD Chest circum-ference Apgar scores	Self-report from multiple interviews First interview contained questions about substance use in yr before preg and during the 1st trimester 2 additional interviews were performed during 2nd trimester and during postpartum hospital stay For the 1st trimester, women were asked about use in the periods before recognition of preg, btwn recognition and diagnosis, and from diagnosis through the 1st trimester Bogus pipeline method used	Use of Cnb, alcohol, and other drugs was assessed per trimester Cnb measured in average daily joints (ADJ) which indicated joints smoked/day for each trimester Hash was estimated as 3 joints per bowl or cigarette and sinsemilla was counted as 2 joints per use	No significant findings were observed for Cnb use and BW, HC, chest circumference, or GA (results for PI were not presented) Cnb use in the 3rd trimester was ss assoc w/ increased BW (see below); however, this was no longer ss in the regression analysis Cnb use in the first 2 mos of the 1st trimester was ss assoc w/ decreased BL. This remained ss in the regression analysis (no statistics were presented for the regression analyses) Mean (SE) growth outcomes for individuals who used average of ≥1 joint/day during preg, compared to non-users: BW (g): Non-user: 3215.0 (26.4) 1st trimester: 3170.0 (38.0) 2nd trimester: 3308.3 (65.2) 3rd trimester: **3357.0 (64.4) p≤0.04** BL (cm): Non-user: 49.4 (0.13) 1st trimester: **48.9 (0.19) p≤0.04** 2nd trimester: 49.7 (0.32) 3rd trimester: 49.9 (0.32) No significant differences in SGA, PTB or minor physical anomalies btwn exposed and unexposed population	Included: Maternal age Education Gravidity Marital status Work status Income Race Preg weight gain Maternal height Tobacco Alcohol Other drugs GA and infant sex were included in all models assessing growth patterns	Heavy Cnb users did not differ significantly from non-users in terms of age and education, but were more likely to be Black and unmarried, and reported higher rates of other substance use during preg Strengths: Measured Cnb use per trimester and calculated frequency Accounted for changing patterns of use in 1st trimester Accounted for variation in the method of Cnb use Limitations: Small sample of users in 2nd and 3rd trimesters Small sample of heavy users Multiple comparisons may have led to a chance finding. Study design excluded women who had little to no prenatal care, which may have limited the no. of heavy Cnb users included

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Day et al. 1992 Pittsburgh, PA MHPCD	Prospective cohort Participants were randomly selected from prenatal clinic at Magee Women's hospital 1982 – 1985 Eligibility criteria: >18 yo >3 mos preg N = 1,360 women interviewed at 4 mo prenatal visit (participation rate = 85%) (same initial sample as in Day et al.,1991) 2 separate cohorts were selected based on stratified sampling. Based on 1st trimester 1st cohort –based on 1st trimester use, women who drank ≥3 alcoholic drinks/wk, and a random sample of women who drank less were selected 2nd cohort – based on 1st trimester Cnb use, women who smoked ≥2 joints/ mo and a random sample of women who used Cnb less often n = 829 participants selected	SGA GA BD Growth and morphology from birth to 3 yrs Weight Length HC PI	Self-report Women were interviewed at 4th and 7th mos gestation and again during postpartum hospital admission Women were subsequently interviewed at each postpartum follow-up about current Cnb use Participant offspring were assessed at birth, 8, 18 and 36 mos	Cnb use was reported per trimester for usual quantity and frequency, minimum and maximum quantity Cnb use frequency was expressed as ADJ Hashish and sinsemilla were converted to Cnb joints in 3:1 and 2:1 ratios, respectively	Cnb use (% reporting use): 1st trimester = 40%, 3rd trimester =18%, 36 mos postpartum = 39% At 8 mos the previously reported assoc btwn prenatal Cnb use and BL was no longer significant No statistics were presented for the any of the following results Current maternal Cnb use at 8 mos was significantly and positively assoc w/ HC There were no other significant assoc btwn prenatal or current Cnb use and growth As reported in Day et al. 1991 - Cnb use in the 1st trimester was ss assoc w/ lower BL. This was no longer ss at 8 mos postpartum -No assoc w/ SGA, GA or congenital anomalies -No significant effect of Cnb use during any trimester on BW, HC, or chest circumference	Included: Maternal age Education Gravidity Marital status Work status Income Race Life events Social support Depression Anxiety Weight gained during preg Maternal height Alcohol Other drugs Tobacco	There were 10 instances of fetal death, 8 of which were mothers who used tobacco during pregnancy. Of these 8, 5 also used Cnb. There were 3 instances of SIDS, all 3 mothers reported heavy tobacco use during pregnancy. Of these, 1 also reported light Cnb use. High percentage of cigarette smokers Strengths: Prospective study w/ frequent follow up assessments Study could control for postnatal exposure Multiple interviews during pregnancy to assess exposure Quantified variation in exposure from different Cnb products Accounted for current Cnb use Could assess tobacco and Cnb together At 8 mos portpartum examined 81% of eligible birth cohort. At 18 mos assessed 90%

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	From birth to 6 yrs, there were 18 deaths, 8 refusals, 16 participants lost to follow up, 21 moved, 1 infant was adopted, and 2 sets of twins n = 763 ANCOVA, stepwise linear regression, logistic regression Separate regression models were performed for Black and White race but no significant difference was seen btwn predictor and outcome variables so race was excluded Interaction tested btwn Cnb, tobacco, and alcohol w/ regression modeling, and contrasting btwn high tobacco and high Cnb compared w/ high tobacco and no Cnb						Limitations: No data were presented for regression analyses of Cnb use and outcomes.

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Day et al. 1994(a) Pittsburgh, PA MHPCD	Prospective cohort Participants recruited from prenatal clinic of Magee Womens hospital, 1983 - 1985 N = 1360 women in 4th mo of preg Based on 1st trimester substance use, women who smoked ≥2 joints/ mo and a random sample of women who used Cnb less often, and women who drank ≥3 alcoholic drinks/wk, and a random sample of women who drank less were selected n = 829 selected and interviewed Women were interviewed again in 7th mo and at delivery n = 668 children examined at 6 yrs Child's environment, including demographic status, SES, medical history of mother and child, psychosocial environment, and nutritional status were also assessed Hierarchical regression, logistic regression	Growth at 6 yrs: Weight Height HC Minor morphologic abnormalities (palpebral fissures, outer canthal distance) Children were assessed at birth, 8 and 18 mos, and 3 and 6 yrs after delivery	Participants were interviewed about Cnb, alcohol, and tobacco use during their 4th and 7th mo gestation, and again during postpartum hospital admission Cnb use assessed for each mo of 1st trimester, then over the entire trimester for remainder of preg 1st trimester measurement was based on weighted average of use from conception to recognition of preg, from recognition to confirmation, and from confirmation to end of 1st trimester	Cnb use frequency was expressed as average daily joints Hashish and sinsemilla were converted to joints at ratios of 3:1 and 2:1, respectively	After controlling for covariates, there was no assoc btwn prenatal Cnb use and growth parameters or minor morphologic abnormalities of offspring at 6 yo Prenatal alcohol exposure was significantly assoc w/ offspring growth at 6 yo (smaller in weight, height, head circumference and palpebral fissure width)	<u>Included in model development:</u> Child sex Child age No. of illnesses Maternal race Maternal height Maternal age Household smoking Nutrition Maternal education Marital status Maternal employment Household income Household structure No. of siblings Life events Maternal depression Maternal hostility Home screening questionnaire Social support Current maternal tobacco, alcohol, cocaine, and other drug use Prenatal tobacco and alcohol use for each trimester	Participants were healthy and of lower SES, 48% were Caucasian and 52% African-American. Cnb use decreased from 40% of sample in 1st trimester to 18% in 3rd trimester. African-American women were more likely to report Cnb use and heavy Cnb use. Heavy users were more likely to be single, and to use alcohol and other drugs. <u>Strengths:</u> Examined offspring at multiple times

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Dekker et al. 2012 Auckland, New Zealand and Adelaide, Australia	Prospective cohort Participants recruited from hospitals or community practices for Screening for Pregnancy Endpoints (SCOPE) study 2004 – 2008 N = 3,234 <u>Inclusion criteria:</u> Nulliparous Singleton preg <15 wks preg at enrollment <u>Exclusion criteria:</u> High risk of PE, SGA, or SPTB Previous knife cone biopsy More than 3 terminations/ miscarriages Current ruptured membranes Known major fetal anomaly or abnormal karyotype Interventions that might modify preg outcome Participants w/ any missing data were excluded from logistic regression, leaving n = 2,499 for SPTB-IM and n = 2,455 for SPTB-PPROM Chi-squared, *t* test, logistic regression	SPTB-IM: spontaneous PTB w/ intact membranes (n=96) SPTB-PPROM: membrane ruptured in the absence of labor, and time btwn rupture to delivery was > 6 hours greater than the time for established labor (n=60) (PPROM is preterm premature rupture of membranes before wk 37 of preg)	Participants interviewed and examined for clinical risk factors at 15±1 wks and 20±1 wks gestation Cnb use recorded for preconception, 1st trimester, and at 15 wks	Participants were categorized as follows: Used Cnb pre-preg (n=213) Used Cnb during 1st trimester (n=41)	<u>SPTB-IM</u>: Assoc w/ pre-preg Cnb, OR = **2.34 (1.22, 4.52)** <u>SPTB-IM:</u> ns assoc w/ Cnb use in 1st trimester (data not presented) <u>SPTB-PPROM</u>: ns assoc w/ Cnb use pre-preg or during 1st trimester (data not presented)	Variables w/ >10% missing data were excluded <u>Included for SPTB-IM:</u> BMI White race Not feeling better than ever History of >1 vaginal bleed Mother w/ type I or II diabetes Mother w/ history of PE Strong family history of LBW Abnormal uterine artery Doppler at 20 wks Shortest transvaginal cervical length <u>Considered for SPTB-IM:</u> 20 other variables, including cigarette use at 1st visit, other drugs in 1st trimester, and maternal age <u>Included for SPTB-PPROM:</u> BMI Height Participant position in family Waking ≥once a	933 variables were tested for assoc w/ SPTB-IM and SPTB-PPROM separately in univariate analysis and excluded if p>0.1. Authors state pre-preg Cnb use may be a more reliable marker of Cnb use because women would be more likely to disclose it than Cnb use during preg. Authors also note that cigarette smoking may not be an independent risk factor for either phenotype because its effect may be explained by other variables in this dataset, such as abnormal uterine artery Doppler.

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						night Mos to conceive Hormonal fertility treatment besides clomiphene Mild hypertension not requiring treatment Family history of GDM Family history of miscarriage Shortest transvaginal cervical length <u>Considered for SPTB-PPROM:</u> 38 other variables, including age, cigarette use at 1st visit	

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El Marroun et al. 2009 Rotterdam, the Netherlands Generation R	Prospective cohort All preg women residing in the study area were invited to participate 2002 - 2006 Enrollment aimed at early preg but was possible until delivery N = 8,880 preg women enrolled Exclusions: use of illicit drugs other than Cnb, missing substance use information, twin preg n = 7,452 Fetal growth parameters were assessed using ultrasound assessments in early (<18 wks), mid- (18-25 wks), and late (≥25 wks) preg Multiple linear regression, longitudinal multilevel analysis Regression models compared Cnb use w/ 2 different reference groups: nonusers and tobacco users	Fetal weight: estimated using femur length and head and abdominal circumference BW HC Trans-cerebellar diameter	Self-report (questionnaires) Timing [asked at enrollment]: Before preg Early preg ("until I knew I was pregnant") Continuing in preg Nonuse Frequency: daily, weekly, monthly	Cnb use only before preg (n=245, 3.3%) Quit Cnb use in early preg (n=173, 2.3%) Continued Cnb use (n=41, 0.55%) Nonuse (no Cnb or tobacco use; n=5540, 74.3%)	Compared to nonuse: <u>Fetal weight</u> <u>in mid-preg</u> Cnb use before preg: ns Cnb use in early preg: ns Continued Cnb use: β = –13.6 (–27.7, 0.1) g <u>Fetal weight in late preg</u> Cnb use before preg: ns Cnb use in early preg: β = **–57.7 (–86.7, –28.7) g** Continued Cnb use: β = **–96.4 (–152.5, –40.4) g** <u>BW:</u> Cnb use before preg: ns Cnb use in early preg: β **= –156.6 (–224.0, –89.2) g** Continued Cnb use: β **= –277.3 (–409.2, –145.4) g** <u>HC in mid-preg</u> Cnb use before preg: β = –0.6 (–1.5, 0.2) mm Cnb in early preg: β = **–1.0 (–2.0, –0.01) mm** Continued Cnb use: ns <u>HC in late preg</u> Cnb use before preg: β = **–1.3 (–2.5, –0.1) mm** Cnb in early preg: β = **–1.8 (–3.2, –0.3) mm** Continued Cnb use: β = –2.5 (–5.2, 0.3) mm <u>Trans-cerebellar diameter</u>: no ss assoc for Cnb or tobacco (data not reported) Smaller ss effects on late preg fetal weight and BW, and	<u>Included</u> GA Fetal sex Maternal age BMI Maternal height Maternal education level National origin Alcohol use Parity Gravidity Psychopathology Cnb and GA interaction term Tobacco use (some models)	Including those who quit smoking tobacco in early preg, 85% of women who used Cnb at some point in preg also used tobacco. The 14.3% of women who did not respond to substance use questions and were excluded were less educated, slightly younger, and less likely to be married than participants. GA was not assoc w/ Cnb or tobacco use.

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					mid- and late preg HC were reported for continued tobacco use Compared to tobacco-only use: <u>BW</u> Cnb in early preg: β = **–95.4 (–168.3, –22.5) mm** Continued Cnb use: β = **–171.7 (–308.3, –35.1) g** <u>Fetal weight in late preg</u> Cnb use in early preg: β = **–40.6 (–71.5, –9.6) g** Continued Cnb use: β = **–67.1 (–124.3, –9.9) g** <u>Fetal weight in mid-preg</u> Cnb effects were ns All Cnb users were pooled to assess <u>dose-response w/ BW:</u> Compared to nonuse Monthly Cnb use β = –123.0 (–263.4, 17.4) g Weekly Cnb use β = **–149.7 (–249.7, –49.7) g** Daily Cnb use β = **–225.7 (–330.7, –120.8) g** When tobacco users were the referent, a dose-response remained but only the assoc for daily Cnb use was ss <u>Fetal weight</u>: Cnb use pre-preg, in early preg, and continued assoc w/ ns, **11.2 (15.3, 7.1) g/wk, and 14.4 (22.9, 5.9) g/wk** reductions,		

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					respectively (nonuse is the referent) HC of fetuses exposed to Cnb before preg, in early preg, and continued in preg grew **0.1 (0.2, 0.02)**, **0.1 (0.2, 0.02)** and 0.2 (0.4, –0.02) mm/wk less, respectively, than fetuses of nonusers Tobacco-exposed fetuses grew 4.1 (5.6, 2.5) g/wk less than nonexposed fetuses, and the reduction in HC growth was also less than in Cnb users (data not reported) Fetal weight: In models w/ Cnb and tobacco as separate but overlapping variables, fetuses exposed to early preg Cnb grew **7.5 (11.65, 3.25) g/wk** less and continued Cnb-exposed fetuses grew **8.9 (17.5, 0.3) g/wk** less, corrected for tobacco exposure. No ss effects of Cnb on HC growth, and no interaction of Cnb and tobacco on fetal growth were found Fetal weight: Compared to fetuses of tobacco smokers, fetuses exposed to Cnb in early preg grew **7.1 g/wk (11.4, 2.8)** less, and those exposed to continued Cnb grew **10.3 (19.0, 1.6) g/wk** less		

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					65.9% of infants of women who continued Cnb use in preg were male, vs. 48.7% among nonusers, p<0.05 Paternal use of Cnb (w/o maternal use) was not assoc w/ fetal growth		

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English et al. 1997 US and Canada	Meta-analysis Search for studies published btwn 1966-1995 10 studies were included of which 7 collected information prenatally N = 32,483 women giving birth to live-born infants (did not specify singleton births) <u>Exclusion criteria</u> Non-English studies Commentaries, letters, and abstracts No adjustment for tobacco Probable confounding No measure of effect reported Pooled estimates (using precision-based weighting) were calculated using fixed effects and random effects models	BW LBW 5 studies examined differences in mean BW 3 additional studies examined rates of LBW 2 additional studies examined both mean BW and LBW	8 studies used self-report alone 1 study used self-report and urine analysis 1 study used urine analysis alone	Studies of mean BW were analyzed as 'any' use during preg One study assessed use per trimester (Day et al. 1991) so pooled estimates were reported for each trimester In order to examine dose-response effect, data from 2 studies (Fried et al. 1984 and Kline et al. 1987) were grouped into infrequent use (≤1 time/wk) and frequent use (≥4 times/wk) Dose response analyses was performed again including 2 more studies, 1 of which used urine assay (presumed to represent immediate and frequent use) and 1 that assessed daily Cnb use	<u>Pooled estimates of BW g, mean difference (CI) assoc w/ Cnb use:</u> 1st trimester: **-48 (-83, -14)** 2nd trimester: **-39 (-75, -3)** 3rd trimester: -35 (-71, 1) (There was substantial heterogeneity among studies, all p values for homogeneity were <0.05) BW analysis was repeated excluding the 1 study (Hingson et al. 1982) that collected exposure data postnatally and may have been subject to recall bias: 1st trimester: - 26 g (-66, 15) (Data for the 2nd and 3rd trimesters were not reported) <u>BW g, assoc w/ frequency of Cnb use, mean difference (CI):</u> ≤1 time/wk: 62 (-8, 132) (test for homogeneity among studies, p=0.59) ≥4 times/wk: -**131 (-209, -52)** (test for homogeneity, among studies, p=0.25) When results from Zuckerman et al. 1989, w/ results of urine analysis, were included in the analysis of frequency of use, mean BW g (CI) reduction was **108 (-139, 50)** Adding the results for each trimester, from Day et	Studies were only included in meta-analysis if they adjusted for tobacco use Studies differed in the covariates controlled for besides tobacco use	Authors state that there was ns assoc btwn Cnb use and LBW; however, they point out that this lack of assoc may be due to the low levels of Cnb typically used by participants in these studies. Analyses of frequency of use was restricted to studies which had data in both 'any' use and frequency of use due to concern that apparent differences in effect according to frequency might be actually due to heterogeneity among studies. <u>Strengths:</u> Multiple analyses were performed to account for various limitations in each study. Results were identical using either fixed or random effects models. <u>Limitations:</u> There was substantial heterogeneity among studies. Authors note that heterogeneity among studies may be due to varying amounts of Cnb used by participants due

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					al.1991, to the analyses of frequency of use: 1st trimester: **-90 (-139, -40)** 2nd trimester: **-78 (-132, -24)** 3rd trimester:**-68 (-122, -14)** <u>LBW</u> (any use, 5 studies included): OR = 1.09 (0.94, 1.27) When data from two studies that collected exposure data postnatally were excluded OR = 1.11 (0.89, 1.40)		to inaccuracy in exposure assessment. Some studies reported use as 'any' use. As authors note this "lessens the ability to examine dose-response effects". Authors note that some studies may have downplayed ns findings, leading to an overrepresentation of ss results in studies. Authors note that the adjustment for tobacco exposure may have been inadequate in some studies.

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Fergusson et al. 2002 Bristol, United Kingdom Avon Longitudinal Study of Pregnancy and Childhood (ALSPAC)	Prospective cohort 1991 - 1992 N = 15,541 preg women from Bristol-based health districts (estimated 85-90% of eligible population) n = 12,129 mothers of singleton pregs >18 wks gestation, who returned the questionnaire ALSPAC staff visited the 2 major maternity hospitals (95% of births) in area to measure BL & HC w/in 24 hours of birth. Detailed data on deaths were abstracted from clinical and postmortem records Chi-square, t test, ANOVA, multiple linear regression	BW BL HC PTB Perinatal death: fetal death ≥20 wks gestation and <7 days after birth NICU admission	Questionnaires were mailed to the mothers at 18-20 wks gestation, asking how often the respondent smoked marijuana/grass/ Cnb/ganja Response options were: Every day 2-4 times/wk 1/wk <1/wk Not at all 90% returned the questionnaire by 25 wks gestation	*Frequency of use in analyses*: Weekly <Weekly Non-user *Timing of use*: Pre-preg use (6 mos before conception) 1st trimester use After 1st trimester *Four combined categories used in growth analyses (n for BW, BL, HC):* 1) Non-user (11464, 7102, 7230) 2) <1/wk both before or throughout preg (294, 184, 185) 3) 1/wk before or during preg but not throughout preg (183, 117, 119) 4) At least 1/wk before & throughout preg (129, 84, 86) 5% of mothers reported smoking Cnb before and/or during preg	Effects of Cnb use vs. no use: BW (g), **p=0.005**: 1) Referent 2) **58.60 (12.91, 165.32)** 3) **89.22 (12.98, 165.30)** 4) −84.20 (−174.70, 6.40) BL (cm), **p=0.004**: 1) Referent 2) 0.04 (−0.25, 0.33) 3) **0.58 (0.19, 0.97)** 4) −0.46 (−0.92, 0.01) HC (cm), p=0.203: 1) Referent 2) 0.067 (−0.13, 0.27) 3) 0.24 (−0.018, 0.51) 4) −0.14 (−0.45, 0.18) BW and BL results were not consistent w/ a dose-response relationship w/ Cnb use during preg Higher BW and BL were assoc w/ Cnb use 1/wk before or during but not throughout preg (3) PTB, perinatal death, NICU admission: not assoc w/ frequency of Cnb use in unadjusted analyses	Included: Parity Tobacco smoking in 1st 3 mos of preg Tobacco smoking at 18-20 wks Alcohol during preg (5 categories) Cups coffee/wk during preg Cups tea/wk during preg Hard drug use during preg Ethnicity Education level Maternal height and weight Sex of child Gestation Considered: Maternal age Tobacco smoking before preg Alcohol before preg	Users were younger; taller; more likely primiparous; more educated; more likely to smoke tobacco; drink alcohol, tea, and coffee; use illicit drugs; and weigh less than non-users (ss in bivariate analyses). Users and non-users were >97% White. Authors note there was little difference in adjusted BW btwn infants of non-users and women using Cnb less than 1/wk before and throughout preg. However, infants of women using Cnb at least 1/wk were ~90 g lighter. Strengths: Use of continuous measurements of outcomes (e.g. mean BW) rather than dichotomous outcomes (e.g. LBW/normal BW) allows researchers to evaluate dose-response relationship Limitations: Low response rate for HC and BL measurements (roughly 50% of the original sample size had available data)

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Frank et al. 1990 Boston, MA	Prospective cohort Participants recruited from prenatal clinics of one hospital 1984 - 1987 N = 1,226 144 excluded due to lack of sufficient data for analysis of infant body composition n = 1,082 Live-born infants were weighed immediately after delivery. W/in 8 to 72 hours, 1 of 5 trained pediatricians who were blinded to exposure status performed anthropometric assessments of infants Periodic inter-observer reliability checks conducted among pediatricians To reduce intra-observer variability in tests, mean values were used in analyses Multivariate regression, ANOVA, Chi- squared and *t*-tests	Neonatal body composition and proportionality Arm circumference Subscapular fat folds Fat and nonfat area of arm PI Arm circumference /HC ratio	Cnb use during preg was measured through interviews and urine assays obtained at least once prenatally, and again postpartum Urine was screened for Cnb and cocaine using enzyme-multiplied immunoassay technique, and confirmed by a second method Cnb analyzed as 2 dummy variables: self-reported use only versus nonuse, and +ve assay result versus nonuse	+ve Cnb urine analysis results (n=171) Cnb self-report only (n=114) No Cnb use (both self-report and assay) (n=797)	Assoc w/ +ve Cnb urine analysis: Arm muscle circumference (cm) **β = – 0.17 (p=0.04**) Subscapular fat fold (mm) β = –0.04 (p=0.71) Fat area (cm^2) β = –0.01 (p=0.94) Nonfat area of arm (cm^2) **β = –0.28** (**p=0.03)** Assoc w/ self-reported Cnb use: PI: **β = 0.07 (p<0.05)** No assoc w/ +ve Cnb urine analysis Arm circumference /HC ratio: no assoc w/ +ve Cnb urine analysis Authors state +ve urine analysis for Cnb and cocaine use was assoc w/ a symmetric pattern of IUGR, which suggests long-term prenatal stress	Included: Infant gender GA Maternal ethnicity Parity Diabetes Cigarette use Opiate use Pre-preg weight for height Preg weight gain Infant gender Maternal diabetes Cocaine use Considered: Alcohol use	Subjects included in study did not differ from the larger sample in drug use or demographic characteristics. Authors suggest assoc btwn self-reported Cnb use and increased PI may be explained by multiple comparisons, or indicates that infrequent Cnb use depresses BL more than BW. Authors suggest that Cnb may retard fetal growth through maternal-fetal hypoxia. Limitations: Authors note that placental weights and microscopic pathologic observations were not documented systematically, therefore conclusions about placental mechanisms of the observed effects are speculative.

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Fried and O'Connell 1987 Ottawa, Canada OPPS	Prospective cohort N = 667 from original sample at birth Most births were in 1980 - 1983 Participants were included if they reported regular Cnb use during preg and were heavier social alcohol drinkers and heavier cigarette smokers n = 123 infants followed up at 1 and 2 yrs Growth data were obtained by assessors who were unaware of the mothers' drug use history Multiple linear regression	At birth and 1 and 2 yrs: Weight Length or height HC	Interview during each trimester Cnb (marijuana and hashish) use was assessed during each interview to validate consistency of self-report	No. of joints used per wk assessed during each trimester Heavy Cnb use defined as 5 joints/wk Mean Cnb consumption among users was 9.09 joints/wk Of 123 children followed to 2 yrs, 41 were prenatally exposed to Cnb	Results for Cnb use were reported only for weight and height at <u>2 yrs</u> Heavy Cnb use in yr before preg ss assoc w/ a **530.17g increase in weight at 24 mos, p<0.01** Heavy use during 1st trimester ss assoc w/ a **539.16g increase in weight at 2 yrs, p=0.02** Heavy use during 3rd trimester ss assoc w/ a **623.16g increase in weight at 2 yrs, p=0.02** Heavy use averaged over entire preg ss assoc w/ a **596.69g increase in weight at 2 yrs, p=0.01** Heavy Cnb use in the yr before preg ss assoc w/ a **0.98 cm increase in height at 2 yrs, p=0.03** Heavy Cnb use over entire preg ss assoc w/ **1.10 cm increase in height at 2 yrs, p=0.05**	<u>Included:</u> Alcohol Caffeine Nicotine Pre-preg weight Preg weight gain Maternal height Parity GA Sex of child Family income <u>Considered:</u> Maternal education	Authors note that prenatal Cnb use counteracts the effects of nicotine use in terms of BW. Authors report that Cnb users tended to consume more protein and calories, which may be reflected in the increased growth of the offspring. Predictors and birth outcomes were similar for subjects who were followed to 12 and 24 mos and those not followed past birth. However, women who used Cnb were more likely to be followed to 12 and 24 mos. <u>Strengths:</u> Multiple interviews. Small loss to follow up. Longitudinal design allowed for direct assessment of fetal growth outcomes and growth at 1 and 2 yrs. <u>Limitations:</u> Limited reporting on outcomes assoc w prenatal Cnb use.

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Fried et al. 1984 Ontario, Canada OPPS	Prospective cohort n = 583 women who delivered single live infants Women volunteered to participate after learning about the study by their obstetrician or notices in public media. Women were interviewed in each remaining trimester about drug use, sociodemographic data, 24 hour dietary recall, and history of previous pregs ANOVA, ANCOVA, Spearman's rank-order correlation	BW Length of gestation (GA), determined by date of last menstrual period, or if unknown, ultrasound and anthropo-metric data	Interviews during each remaining trimester of preg about maternal Cnb, smoking, and alcohol use Because Cnb use across trimesters was highly correlated, avg Cnb analyses used across preg	Categories based on # joints/wk Nonusers (n=499) Irregular users: Smoked avg ≤1 joint/wk (n=48) Moderate users: smoked 2-5 joints/wk (n=18) Heavy users: smoked >5 joints/wk (n=18)	GA (wks): Mean (SD) Nonusers: 39.6 (1.67) Irregular users: 40.2 (1.19) Moderate users: 40.2 (1.83) Heavy users: 38.8 (1.77) **p-value=0.008** (ANCOVA) Mean GA of infants of heavy users shorter than nonusers by 0.8 wks (**p=0.039**) Among heavy users, a dose-dependent relationship btwn shortened GA and amount of Cnb smoked was observed (Spearman's correlation **r=-0.401, p=0.050**, one-tail) BW: Mean BW of heavy users was 52 g less than mean BW of nonusers (p=0.598, from Table II [text reports 78 g difference, ns])	Included for GA: Maternal pre-preg weight Included for BW: Maternal age Pre-preg weight Nicotine use Sex of infant Considered: Alcohol Parity Family income Maternal education	The 18 heavy users were distributed as follows: 6-12 joints/wk (n=5) 13-19 joints/wk (n=6) 20-50 joints/wk (n=4) >50 joints/wk (n=3) Of the 84 Cnb users, 4 reported using LSD or cocaine, and 2 reported amphetamine use, but none reported other drug use past the 1st trimester. Urine samples taken for all moderate or heavy users in the 3rd trimester interview screened negative for opiates and amphetamines Significant differences in income and level of education between moderate/heavy users and nonusers/irregular users

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Fried et al. 1999 Ottawa, Canada OPPS	Prospective cohort Most babies born 1980 - 1983 <u>Eligibility criteria:</u> Delivered after 36 wks gestation Singleton birth No physical or chromosomal anomalies N = 698 women interviewed during preg n = 679 at birth Follow-up cohort: 140 children of women who reported any Cnb use, drank daily avg >0.85 oz AA, or smoked ≥16 mg nicotine/d, and 50 randomly selected children of women who used no Cnb or nicotine, and drank little or no alcohol Follow-up sample sizes ranged from n=117 at 4 yrs to n=201 at 1 yr ANOVA, ANCOVA No adjusted analyses for height or HC at birth were presented	Birth outcomes and postnatal growth assessed at birth, 1, 2, 3, 4, 6 and 9-12 yrs Weight Height HC Standard scores (z-scores) for the outcomes were calculated for boys and girls separately using norms for weight and height to 12 yrs and for HC to 3 yrs from the National Center for Health Statistics	Self-report by in-person interviews performed several times throughout preg In some instances women who delivered earlier than expected were interviewed immediately postpartum	Cnb use averaged across preg was categorized into 3 groups: No use Mild/moderate use (<6 joints/wk) Heavy use (≥6 joints/wk)	Adjusted Z-score (SE) for outcomes at each age, assessment for each joints/wk category (ns unless p-value shown): <u>Weight:</u> Birth (adjusted for maternal cigarette and alcohol use, GA, parity): No use: 0.67 (0.04) Moderate: 0.53 (0.12) Heavy: 0.53 (0.18) 1 yr: No use: -0.07 (0.08) Moderate: -0.28 (0.17) Heavy : 0.33 (0.26) 4 yrs: No use: -0.05 (0.09) Moderate: -0.05 (0.18) Heavy: 0.48 (0.21) <u>Height</u>: No adjusted data reported. Authors report ns greater height in heavy users' children at 1-4 yrs. No significant differences across Cnb use categories <u>HC</u> (adjusted for maternal cigarette and alcohol use): 6 yrs: No use: 0.94 (0.13) Moderate: 1.06 (0.24) Heavy: 0.49 (0.28) 9-12 yrs: No use: 1.41 (0.13) Moderate: 1.93 (0.26) Heavy: 0.83 (0.28), **p≤0.05**	<u>Considered:</u> <u>All ages</u> Family income Maternal age Nonpreg weight Maternal height Other drug use Alcohol Tobacco Paternal height Paternal weight <u>Birth</u> GA Parity Maternal nutrition Prenatal caffeine Parental health history Preg difficulties <u>1 yr</u> Gestation length Parity Method of feeding Breast-feeding duration	Compared w/ controls, Cnb users were younger, had lower family incomes, and had shorter gestation <u>Strengths:</u> Accounted for frequency of Cnb use Authors state repetition of interviews by same female interviewer enhanced rapport w/ subjects and permitted evaluation of the consistency of self-report During first 6 yrs of life, offspring were measured w/in 1 mo of each birthday Accounted for detailed alcohol and tobacco exposure <u>Limitations:</u> Diminishing sample size w/ each follow up Multiple comparisons

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					Daughters of heavy Cnb users were lighter than sons at 1 yr after adjustment for maternal cigarette and alcohol use ($p<0.05$)		

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Fried et al. 2001 Ontario, Canada OPPS	Prospective cohort N = 698 women from original OPPS cohort 1979 - 1985 From original sample, 140 children of women who reported any Cnb use, drank daily avg of >0.85 oz AA, or smoked ≥16 mg nicotine/d, and 50 randomly selected children of women who used no Cnb or nicotine, and drank little or no alcohol comprised the follow-up cohort. n = 152 offspring followed to 13-16 yrs (83 boys, 69 girls). Included participants were singletons who were delivered at ≥36 wks gestation, and were willing and available for testing. ANOVA w/ Scheffé test, t test, ANCOVA	Growth measure-ments at 13-16 yrs: Weight Height HC PI Pubertal development: Menstruation Breast development Shaving Voice change	Interviews during each trimester about maternal drug use amount and pattern	Categories based on no. of joints/wk: No use (n=101) Infrequent or moderate use, >0 and <6 joints/wk (n=26) Heavy use, ≥6 joints/wk (n=25)	Results are unadjusted, except as noted Growth measurements: No differences for weight, height, PI. Heavy use was marginally assoc w/ decreased HC (p=0.08), but this relationship was weaker when prenatal alcohol and nicotine exposure were considered (p=0.11). Pubertal development: No differences across levels of Cnb exposure Maternal Cnb use did not affect offspring growth differentially by sex	Considered but not used in Cnb analyses because all ANOVA results for Cnb exposure and each outcome variable were ns: Prenatal alcohol Prenatal tobacco Family income at birth Maternal age at delivery Pre-preg weight, height Pre-preg other drug use Paternal weight and height BW Present family income Current adolescent tobacco	No differential loss of subjects w/ respect to drug variables Authors state that prenatal Cnb use was significantly, linearly assoc w/ HC when subjects were 9-12 yo, and the trend was evident though ns at each of the earlier ages (birth, annually until 4 yrs, 6 yrs) assessed in OPPS. Limitations: Study only reported adjusted results for HC.

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Gibson et al. 1983 Adelaide, Australia	Prospective cohort Participants recruited from the Queen Victoria Hospital or Queen Elizabeth Hospital 1975 – 1981 N = 7,725 Prenatal interview collecting demographic information and drug use history, performed upon study entry 2nd interview, during admission for delivery, for drug use during preg Participants excluded for multiple births and having incomplete information on drug use n = 7,301 Information on the preg, labor, and neonate compiled from various hospital records by an obstetrician Logistic regression	LBW IUGR (diagnosed by neonatologist, considering condition and progress of the baby, maternal antenatal conditions, ultrasonic biparietal measure-ments) PTB BD Perinatal death: infant stillborn or died w/in 30 days of birth Low Apgar score: ≤4 at 1 min test	Self-report by interview	Cnb use classified as: Nonuser (n=6,909, 94.6%) ≤Once/wk (n=356, 4.9%) >Once/wk (n=36, 0.5%) 91.6% of Cnb users also used alcohol or tobacco. Only 33 women used Cnb alone.	LBW: >once/wk Cnb was assoc w/ increased risk **(p=0.011),** but was ns when PTB infants were excluded from analysis (p=0.26) IUGR: Heavy prenatal Cnb use ns assoc w/ increased risk (p=0.12). Only 72 (0.99%) infants met the case definition PTB: any Cnb use assoc w/ increased risk, **p=0.002** No. (%)±SE of PTB in each level of Cnb use: Nonuser: 384 (5.6)±0.28 ≤Once/wk: 18 (5.1)±1.17 >Once/wk: 9 (25)±7.22 Excess risk assoc w/ PTB is due to high proportion of PTB among women who reported using Cnb >once/wk. BD, Low Apgar score: No significant assoc w/ Cnb use Perinatal death: "suggestion (p=0.12) of an association" w/ Cnb use. 66 of 91 perinatal death cases were also PTB and were included in the analysis	Included: Maternal age Parity Alcohol Tobacco	Authors note that information on type of Cnb use not always available but most regular users (≥2 times/wk) used more potent forms (hash and "Buddha sticks"). 40% of the sample smoked and 68% drank approximately ≤1 alcoholic drink/day. Authors note that though Cnb was ns assoc w/ IUGR, the low no. of heavy Cnb users may have affected the ability to achieve significance and further study is suggested. Strengths: Stratified by levels of use. Limitations: Small no. of frequent Cnb users.

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Gray et al. 2010 Buffalo, NY	Prospective cohort The source of the study population and the yrs of subject selection were not specified. N = 120 (preg women btwn 12 and 20 wks gestation met eligibility critieria) <u>Exclusion criteria</u>: Maternal age <18 yo, Multiple-birth pregs Illicit drug use other than Cnb Heavy alcohol or Cnb consumption (>1 drink or >5 joints/day or >4 drinks or >4 joints on a single occasion) 16 women lost to follow up, 1 suffered miscarriage, 1 relocated n = 102 n = 86 after sample collection T tests, Mann-Whitney U test, Spearman ρ test Chi-squared test	BW BL HC GA Apgar scores	Prenatal assessment at end of each trimester, and approximately 2 mos postpartum "Time-line" follow-back interview at each assessment gathered retrospective data on daily tobacco, alcohol, and Cnb use for previous 3 mos and 3 mos pre-conception Oral fluid sample at each assessment analyzed by immunoassay screening and GC-MS for Cnb (This assay has a short detection time window. Thus, the presence of THC indicates recent maternal Cnb use) Cotinine analyzed by ELISA or liquid chromatography-tandem mass spectrometry Meconium samples analyzed by GC-MS Meconium also analyzed for nicotine exposure	If Cnb use was reported in grams, blunts or bowls, participant were asked how many joints could have been rolled w/ the quantity of Cnb. If unknown, bowls and blunts were recorded as 3 and 4 joints, respectively Cnb users = self-reported, or positive oral fluid or meconium results	Based on combination of self-report and oral fluid testing: 53.5% had no Cnb exposure, 18.6% smoked only in the 1st trimester, 9.3% stopped in the 2nd trimester 18.6% smoked throughout preg No. of joints smoked per trimester, median (IQR): 1st = 70.8 (11.11-308) 2nd = 30.2 (1.9-170) 3rd = 5.8 (1.8-7.4) Results based only on meconium, independent of tobacco status, mean (SD): <u>BW (g):</u> Cnb -ve: 3429 (544) Cnb +ve**:** 2856 (618) **p=0.001** <u>BL (cm)</u>: Cnb -ve: 50.8 (2.4) Cnb +ve**:** 48.8 (4.4) **p=0.010** <u>HC (cm):</u> Cnb -ve: 34.4 (1.7) Cnb +ve: 33.0 (2.4) **p=0.003** <u>GA (wks):</u> median (IQR) Cnb –ve: 39 (38.7, 40.2) Cnb +ve: 39 (37.2, 39.5) **p=0.012** Results based only on meconium, in tobacco +ve mothers remained significant for BW, BL, HC but not for GA	<u>Included:</u> Race – White GA Cnb +ve meconium Tobacco +ve meconium <u>Considered:</u> Race Marital status Maternal age Obstetric history Employment status	Significant differences in race and concurrent tobacco consumption were observed btwn Cnb users and nonusers. Hispanic women consumed the least Cnb, African-American women consumed the most Cnb. More neonates were identified as Cnb exposed by mother's self-report than by meconium analysis. Authors note that because many women quit Cnb use after the 1st or 2nd trimester, a positive meconium sample is more likely if Cnb use is continued into the 3rd trimester. Authors reported that fetal growth restriction was assoc w/ positive meconium samples, but not by self-reported Cnb exposure. Thus, the effects of prenatal Cnb exposure may be more severe if exposure occurs in late preg or for the duration of the preg. There was no correlation btwn cannabinoid biomarker concentrations or no. of reported joints smoked during the 1st

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					Results based on +ve self-report, oral fluid sample, or meconium, independent of tobacco remained significant only for BW and HC Results based on +ve self-report, oral fluid sample, and meconium, in tobacco +ve mother were ns for comparisons btwn Cnb users and nonusers Regression analysis for BW reported only the following variables impacted the model's ability to predict BW as follows: (R^2=0.532), GA (β=0.568), race/White (β =0.272), Cnb +ve meconium (β = -0.229) There were no ss results for any comparisons of 1-min or 5-min Apgar score		trimester and any outcome measures. Strengths: Examined Cnb use per trimester, and analyzed specific effects of each trimester's use. Used 3 methods of exposure assessment w/ a sophisticated self-reported quantification method. Analyzed results of meconium positive samples only, as well as results of meconium, self-report and oral fluid. Limitations: Oral fluid samples can only detect use w/in 24 hours. Infrequent sample collection may underestimate prevalence. There was no relationship btwn maternal self-reported no. of joints consumed in the 3rd trimester and meconium biomarker concentrations. This may be due to inaccuracies in self-reporting, variation in THC concentration, timing of exposure or different methods of Cnb smoking.

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Greenland et al. 1982 Los Angeles, CA	Prospective cohort Prenatal clinics of UCLA Center for Health Sciences and Kaiser Permanente n = 71 women (35 users & 36 non-users) <u>Inclusion criteria:</u> <30 wks gestation at enrollment 18-35 yo No major medical problems (eg. diabetes, hypertension) Either used Cnb >1x/mo (users) or no use at all (non-users) w/in 3 mos of conception or during preg <u>Exclusion criteria:</u> Used Cnb ≤once/mo; co use of other illegal drugs Fischer's exact test	Abnormal fetal test (any type) Apgar score Precipitate labor Prolonged, protracted, or arrested labor Manual removal of placenta Respiratory distress syndrome (RDS) Resuscitation Meconium staining *data on LBW and GA were collected but not analyzed	For most results, self-report (use >1/mo or no) For screening of recent use at entry and at delivery, maternal blood, cord (fetal) blood, and maternal urine were collected Samples underwent radioimmunoassay to test for ∆9-THC Other data ascertained from medical records First interview after entry to study looked at: drug use, medication, tobacco, alcohol, dietary habits, preg history Blood and urine samples taken at first interview Second interview ~10 wks after to note current habits and review updated medical records Third interview 'shortly after delivery' to assess drug use and 'other events'	Nonusers: no use during preg or 3 mos prior to conception Users: ≥once/mo during preg In several cases, a matched nonuser was not available so the ethnicity criteria was relaxed	Cnb users: ns higher levels of anemia, poor weight gain, 'suspected' intrauterine growth retardation (IUGR), and prolonged/protracted/ arrested labor, abnormal fetal tests and manual removal of placenta Precipitate labor was higher among users (29% vs. 3% non-users, **p<0.01;** unadjusted) Higher proportion of users had meconium staining (57% users vs. 25% non-users; **p=0.05;** unadjusted) Matching variables were accounted for Single variable adjustment for ethnicity, income, smoking, alcohol use, first physician visit did not meaningfully change results (other than ethnicity, data not presented) Significant difference in precipitate labor for users w/ positive THC test at delivery compared to users testing negative (**p<0.01**) Outcomes like RDS were too infrequent to use for analysis	<u>Matched:</u> Nonusers matched to users on: Age Previous preg status Ethnicity Center of care <u>Included</u> (singly, in sensitivity analyses): Ethnicity Income Smoking Alcohol use First physician visit	Higher proportion of Cnb users consumed alcohol and tobacco compared to nonusers. <u>Limitations</u>: Small n for users (and nonusers). Authors explain that their strict criteria for Cnb use resulted in a limited no. of eligible users. Although chance can contribute to statistical significance, observed trends were consistent w/ literature and animal studies.

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Greenland et al. 1983 Los Angeles, CA	Prospective cohort n = 313 Participants were 1st women who enrolled for home deliveries at the Los Angeles Childbirth Center Inclusion criteria: intent to have home delivery attended by nurse-midwife, resident in West LA, low risk medical status established by physician/nurse-midwife team and maintained to the 36th wk Fisher's exact test, logistic regression (for dose-response)	Maternal: Premature rupture of membranes prior to labor (PPROM) Primary dysfunctional labor Precipitate labor Manual removal of the placenta Secondary arrest of labor Labor ≥20hrs Prolonged latent phase of labor Forceps use Cesarean Postpartum hemorrhage ≥500mL Neonatal: Meconium in amniotic fluid LBW Resuscitation Jaundice	Cnb exposure assessed by self-report to the physician and/or nurse-midwife (smoke only, other forms not recorded)	Nonusers (n=272): no Cnb use at all during preg Users (n=41): self-reported use any time during preg Frequency measured in joints/mo Reported use typically constant across preg Further quantified in 3rd trimester: ≤once a wk (n=9) >once a wk but <once a day (n=19) ≥once a day (n=12)	Slightly higher frequency of problems in labor and delivery, most ns. outcomes for users vs. nonusers (CIs for differences); nearly all ns: PPROM 23% vs. 19% (-10%, 18%) Primary dysfunctional labor 43% vs. 35% (-8%, 24%) Precipitate labor 13% vs. 8% (-6%, 16%) Meconium staining 17% vs. 13% (-8%, 16%) Manual removal of placenta 10% vs. 3% (0%, 17%; **p<0.05**) Frequencies and rates for other outcomes were reported in tables w/o significance tests; differences presumably ns Ns dose-response relationship btwn dysfunctional labor and smoking ≥30 joints/mo: 3% increase in risk per joint smoked/mo in the 3rd trimester, adjusted for age Insufficient nos. to analyze dose-response relationships for other outcomes Confounder-adjusted results not reported, but changed results "in only a trivial fashion"	Considered (in sensitivity analyses): Alcohol Cigarette use Parity Income Maternal age (tested singly by standardization)	Physicians and nurse-midwives developed close relationships w/ women in study, which authors state supports strength of self-report. Blinding was not utilized. Limitations: Relied on self-reporting, but authors state drug use patterns in this study are consistent w/ Greenland et al. (1982), which screened blood and urine for recent Cnb use

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Gunn et al. 2016 US, Canada, Australia, the Nether-lands, Iran, Jamaica, Spain, Brazil	Meta-analysis Included: 1 case-control, 1 cross sectional, and 22 cohort studies Systematic review: 6854 articles were screened (results from searches in 7 major databases) 24 articles were selected for analysis (inclusion criteria - only studies that reported outcomes of Cnb use while excluding other illicit drug use during preg; English only) Meta-analysis continuous data: outcomes were extracted as means, SDs. Fixed-effect models were used when heterogeneity was acceptable (Q test - p>0.10, or p≤0.10 and I^2≤50) Random-effects model was used when heterogeneity was not significant (Q test - p≤0.10, but I^2>50%) Results were pooled for each outcome	BW (10 studies) LBW (7 studies) BL (9 studies) HC (10 studies) SGA (2 studies) GA (5 studies) PTB (9 studies) NICU admission (4 studies) Placental abruption PROM Other birth outcomes: See results Maternal outcomes: See Results Apgar scores (6 studies)	18 studies used self-report to assess exposure, 6 used urine screening, 3 used meconium, 2 used serum analysis, 1 used maternal hair samples	This study did not specify each study's exposure quantification Exposure was dichotomized for analysis and categorized infants as exposed or unexposed	Pooled estimates (95% CI) for studies that examined prenatal Cnb exposure for each outcome (pOR (pooled OR), pMD (pooled mean difference): BW: pMD = **109.42g** decrease in BW **(38.72, 180.12)** I^2=63% LBW: pOR = **1.77 (1.04, 3.01)** I^2=89% BL: pMD = -0.10 (-0.65, 0.45) I^2=59% HC: pMD = -0.31 (-0.74, 0.13) I^2=97% PTB: pOR = 1.29 (0.80, 2.08) I^2=85% SGA: ns (estimate not reported) Apgar scores: ns (estimate not reported) NICU admission: pOR = **2.02 (1.27, 3.21)** I^2=78% GA: pMD = -0.20 (-0.62, 0.22) (I^2=33%) No significant assoc found btwn prenatal Cnb exposure and SAB, placental abruption, days in the hospital, jaundice, resuscitation, respiratory distress syndrome, perinatal mortality, fetal distress, BD, abnormal fetal tests, blood transfusions, needing intubation after delivery,	Studies were excluded if they did not account for illicit drug use, tobacco use, or alcohol	Strengths: Meta-analysis allows for substantial increase in sample size. The study assessed risk of bias using quality assessment tools (for cohort and case-control studies used - Critical Appraisal Skills Programme-Making Sense of the evidence was used; for x-sectional studies - National Collaborating Centre for Environmental Health was used). All the studies were determined to be of high quality. All studies scored btwn 7 or 8 out of a possible score of 8 (supplement 2). Limitations: No control for potential confounding variables such as tobacco or alcohol use. Authors noted that as many Cbn users are often tobacco or alcohol users, "determining a Cbn-only effect (excluding the presence of tobacco and alcohol) was currently not possible in this systematic review and meta-analysis with the available literature".

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					hypoglycemia, or sepsis, chromosomal abnormalities Maternal anemia: pOR = **1.36 (1.10, 1.69)** No significant assoc were found w/ maternal Cnb use and maternal diabetes, rupture of the membranes, premature onset of labor, prolonged labor, dysfunctional labor, prenatal care, duration of labor, secondary arrest of labor, elevated blood pressure, hyperemesis gravidarum, maternal bleeding after 20 wks, ante/postpartum hemorrhage, maternal weight gain, maternal postnatal problems, days in the hospital, or hormone concentrations		Each exposure assessment strategy has limitations, meaning combining various strategies may distort true estimates. This study could not restrict maternal age to ≥18 yo as some studies did not report maternal age and too many studies would have been excluded. A sub-group analysis could not be conducted when heterogeneity was >50% due to the limited no. of studies that were included. No sensitivity analysis or use of funnel plots.

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Hatch and Bracken 1986 New Haven, CT	Prospective cohort Participants from New Haven area presenting for 1st prenatal visit intending to deliver at Yale-New Haven Hospital 1980 - 1982 N = 6,219 n = 3,857 singleton live births w/ valid, completed interviews, after exclusions, loss to follow up, missing information Shortly after 1st prenatal visit, women were interviewed in their homes about demographics, medical history, other risk factors Chi-square tests, linear and logistic regression	Obtained from medical records: BW LBW SGA (BW <10th percentile for race, sex, mother's parity, and GA) GA PTB	Women were asked whether they had used Cnb or hashish since becoming preg and frequency of use during preg. Possible answers shown on a response card ranged from <once/mo but once since preg to ≥5 times/day	Frequency of Cnb use: No use (n=3,490, 90.5%) Occasional (≤ once/mo, n=158, 4.1%) Regular (≥2-3 times/mo, n=209, 5.4%) Of the regular users, 43 reported Cnb use 1-5 times/day Majority of occasional users had used only once since conception	Occasional Cnb use: no excess risk of LBW, SGA, or PTB Evidence for interaction btwn race and Cnb use for LBW (p=0.06) and SGA (p=0.01), so separate models were fit for Whites, non-Whites, and both LBW OR for regular vs non-use: Whites **2.6 (1.1, 6.2)** Non-Whites 0.7 (0.6, 1.8) SGA OR for regular vs non-use: Whites **2.3 (1.3, 4.1)** Non- Whites 0.6 (0.2, 1.6) PTB OR for regular vs non-use: All races 1.5 (0.9, 2.5) Whites* 1.9 (1.0, 3.9) *Interaction btwn Cnb use and race was ns, OR not reported for non-Whites BW (all races), compared to non-use: Occasional use β=1 g Regular use β=–44 g p=0.40 Although interaction btwn Cnb use and race was ns, authors report: BW (Whites): Occasional use β=–24 g Regular user β=–97 g, p=0.07	Included as indicated by subscript: Parity[1] Cigarettes/day in 1st mo of preg[1,2] GA[1] Education[3] Race[3] Caffeine (mg/day)[4] [1] LBW [2] SGA [3] PTB [4] BW Considered: Maternal age Marital status Prior SB or induced or SAB Alcohol (ml/day) Interaction terms for Cnb w/ race, alcohol (ns), and cigarette use (ns)	Authors suggest possibly greater misclassification of Cnb use and other risk factors among non-Whites could explain lack of effect in this group, or that higher risk of adverse outcomes makes the effect of Cnb use more difficult to detect. ORs for regular Cnb use and LBW and SGA were greater than ORs for smoking ≥20 cigs/day.

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Hingson et al. 1982 Boston, MA	Retrospective cohort 1977 – 1979 N = 3,222 women who delivered singleton infants in Boston City Hospital n = 1,343 to 1,384 mother-child pairs w/ interview and infant physical examination included in analyses 1,690 mothers were interviewed; main reasons for no interview: refusal, language, not contacted (mainly due to discharge prior to contact) Newborn physical examination by physicians who were unaware of interview results No mention of why some infants were not examined Multiple and logistic regression	BW BL HC GA <u>Congenital abnormality</u> defined as ≥3 minor malformations or ≥1 major malformation Features compatible w/ fetal alcohol syndrome (CFAS)	Structured interview about variables thought to influence fetal development, diet, health history, and alcohol and tobacco use, conducted in English or Spanish by a female interviewer after delivery Cnb use was assessed as never, prior to preg only, prior to and during preg, and for each trimester 14% of mothers interviewed reported Cnb use during preg	Cnb use during preg was classified as follows (n=1690): Never (86%) <1 per/mo (3%) Once/mo but < once/wk (3%) 1-2 times/wk (6%) 3+ times/wk (2%)	Frequency of Cnb use during each trimester was too correlated to identify when Cnb use had an effect <u>BW:</u> assoc btwn maternal Cnb use during preg (compared to non-use): Any: **–105 g (p<0.01)** <3 times/wk: **–95 g (p<0.01)** ≥3 times/wk: **–139 g (p<0.01)** <u>BL, HC, GA, congenital abnormality:</u> assoc w/ Cnb use were not reported <u>CFAS</u>: Women who smoked Cnb during preg "were **five times more likely than nonusers (2.0, 12.7)** to deliver a child w/ CFAS features (*P* < .001)", whereas drinking ≥2 drinks daily compared w/ nondrinkers was assoc w/ ns reduced risk	<u>Included</u>: Log GA (in growth regressions) Cigarettes/day during preg Black race Age at preg Pre-preg weight Preg weight change Sex of child Alcohol use Psychoactive drug use (maternal, paternal) prior to and during preg Coffee Vitamins Education Maternal height Previous miscarriages, abortions Nutrition during preg X-ray during preg Paternal alcohol use Serious maternal risks (e.g. accident, toxemia) Illness prior to or during preg	No. of subjects and covariates included were not reported for every analysis. 78% of infants received a physical examination. Mothers of babies who were not examined were less likely to have received prenatal care, but were otherwise similar to mothers of babies who were examined. 67% of women whose infants were examined were interviewed Mothers were mostly young, low-income, Black Smoking ≥1 pack/day during preg was assoc w/ 83 g (p<0.01) lower BW compared w/ no cigarette smoking. Among a subset of 328 English-speaking women who were interviewed both at a prenatal visit and after delivery, 15% and 18% of women reported prenatal Cnb use, respectively. Only 36 of these women reported prenatal use in both interviews, while 72 reported in either interview. Using data from prenatal interviews, preg Cnb use was not related to

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							infant size but was weakly assoc w/ shorter gestation (p=0.07).

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Howard et al. 2019 Cincinnati, OH	Retrospective cohort 2013 - 2014 N = 3,310 women who delivered singletons at a large tertiary academic referral center where the standard of care was for all patients to have a urine drug screen at presentation for prenatal care and at admission for delivery Participants excluded if one of the urine drug screens was not available, or if one was +ve for drugs other than Δ^9-THC n = 2,173 Maternal, preg, and infant data abstracted from electronic medical record Mann-Whitney *U* test, chi-squared, Fisher exact tests, ANCOVA	BW BL HC GA Apgar scores Neonatal abstinence syndrome Perinatal mortality	Urine sample collected at 1st prenatal visit, as well as at admission for delivery Meconium screening performed for any infant whose mother had a +ve screen for any drug at delivery Meconium samples were collected for 765 infants	Exposure categorized as follows: –ve results for both drug screenings (n=1683) +ve at initial screening only (n=348) +ve at delivery only (n=27) +ve for both screenings (n=115) 490 (22.6%) of mothers tested +ve at some point in preg	Medians and p-values for unadjusted comparison to Cnb –ve (p), ANCOVA p-adjusted for tobacco (p_T): BW (g) Cnb –ve: 3235 Cnb +ve initial: 3160 **p=0.009,** p_T=0.089 Cnb +ve delivery: 2785 **p<0.001, p_T<0.001** Cnb +ve both: 2925 **p<0.001, p_T<0.001** BL (cm) Cnb –ve: 20.1 Cnb +ve initial: 19.9 **p=0.001,** p_T=0.383 Cnb +ve delivery: 19.0 **p<0.001,** p_T=0.050 Cnb +ve both: 19.1 **p<0.001, p_T<0.001** HC (cm): Cnb –ve: 13.4 Cnb +ve initial: 13.4 **p=0.049,** p_T=0.483 Cnb +ve delivery: 13.2 p=0.261, p_T=0.185 Cnb +ve both: 13.2 **p<0.001,** p_T=0.076 GA (wks): Cnb –ve: 39.3 Cnb +ve initial: 39.2 p=0.885, p_T=0.662 Cnb +ve delivery: 39.0 p=0.062, **p_T=0.008** Cnb +ve both: 39.0 **p=0.012, p_T<0.001**	Included: Tobacco	Patients who screened +ve for Δ^9-THC tended to be younger and were more likely to be African American than those who did not. Results suggest that Cnb use during 3rd trimester and use throughout preg have greatest effect on BW and BL. Strengths: Did not rely on maternal self-report, used two urine screenings. Analyzed findings for all screenings. Large Cnb use sample. Excluded other drug use. Limitations: Urine screenings have a brief detection window. Did not account for alcohol use or other potential confounders.

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					No significant differences in 1 or 5-min Apgar scores and neonatal abstinence syndrome btwn Cnb +ve and Cnb –ve Perinatal mortality: n (%) Cnb –ve: 18 (1.1) Cnb +ve initial: 4 (1.1) p=0.781 Cnb +ve delivery: 1 (3.7) p=0.262 Cnb +ve both: 5 (4.3) **p=0.013**		

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Hurd et al. 2005 Brooklyn, NY	Retrospective cohort n = 139 (44 Cnb users and 95 non-users) 2000 - 2002 Participants were women who had elected saline-induced abortion, recruited at mid-gestation (17-22 wks) Participants completed a structured verbal questionnaire for demographic and drug use information, and provided urine at recruitment Fetuses included if postmortem interval ≤24 hours Mothers ≤15 yo and who used other drugs were excluded Meconium was collected and fetuses were examined and measured Generalized linear modeling regression	Mid-gestational fetal growth: Weight Foot length Body length Occipital-frontal HC PI	Cnb use prior to and during preg was assessed by interview Maternal urine screening: 50 ng/ml considered +ve Meconium screening: immunoassay 10 ng/g considered +ve; 2nd analysis using GC-MS Subjects considered exposed to Cnb if +ve urine and/or meconium, and/or +ve self-report A blunt was counted as 3 joints	Dichotomized variable based on self-report or urine/meconium screening Continuous variables: Joints/use Frequency (times/wk) Categories: Nonuse Light use (>0 to <0.4 average joints/day (AJD)) Moderate use (≥0.4 to <0.89 AJD) Heavy use (≥0.89 AJD) 31.7% of participants were +ve for Cnb use based on self-report or urine/meconium screening	Use *during* preg vs. non-use: Weight: **–14.53 (– 28.21, 0.86) g; *p*=0.04** (as reported) Foot length: **–0.08 (–0.15, –0.01) cm; *p*=0.02** Body length: –0.05 (–0.41, 0.52) cm HC: –0.07 (–0.42, 0.28) cm PI: –0.03 (–0.09, 0.02) Assoc w/ Cnb use *before and during* preg vs. non-use Weight: –10.41 (– 22.59, 1.77) g; *p*=0.09 Foot length: **–0.08 (–0.14, –0.01) cm; *p*=0.02** Body length, HC, PI: no ss assoc Assoc for amount (AJD) and frequency of Cnb use during preg Weight: Amount –9.84 (–21.05, 1.38), p=0.09 Freq –34.98 (–80.32, 10.36) Light use –15.09 (–53.71, 23.52) Moderate use **–54.19 (–103.59, –4.78)** Heavy use –22.17 (–65.90, 21.54) Foot length: Amount **–0.08 (–0.14, –0.02)** Freq **–0.27 (–0.50, –0.03)** Light use –0.10 (–0.30, –0.10) (as reported) Moderate use **–0.28**	Included Alcohol use Cigarette use GA Fetal sex Maternal age Maternal education Marital status	30% refusal rate High proportion of Cnb users Cohort was predominantly black and unmarried, and generally similar for Cnb users and non-users Strengths Self-report and urine and meconium screening for Cnb, cocaine, opiate, amphetamine use Analysis of Cnb use as a continuous variable Limitations Many inconsistencies in text and tables

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					(–0.54, –0.02) Heavy use –0.20 (–0.43, 0.03) Body length, HC, PI: no ss assoc		

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Janisse et al. 2014 Detroit, MI	Prospective cohort Participants were enrolled at initial prenatal visit at an urban tertiary care center All women who reported ≥ 0.5 oz absolute alcohol consumption/day, and a random sample of 5% of women who reported abstaining or light alcohol consumption were invited 1986 – 1998 Eligibility criteria: African-American Ultrasound-supported estimate of GA available Exclusions: Fetal anomalies Induced labor for reasons other than PROM N = 3,164 n = 3,090 for path analysis Sociodemographic and clinical data collected at initial prenatal visit, infant data collected at birth Path analysis to test direct effects of predictors on GA and growth as mediators of the effect on decreased BW	BW Fetal growth (represented by BW residualized for GA, as a z-score, normalized separately above and below the median) GA	Assessed by detailed interview at each prenatal visit	Cnb use quantified as proportion of prenatal visits in which the woman reported use, grouped into four categories: No use (75.8%) 1-33% of visits (15.3%) 34-66% of visits (6.3%) 67-100% of visits (2.6%) 24.2% used Cnb	BW: High prenatal Cnb exposure alone (defined as Cnb use reported in >33% of prenatal visits, w/ no other drug exposure) compared to no use was assoc w/ **55 g lower BW** (attributed to reduced fetal growth, statistics not reported) Fetal growth: Prenatal Cnb use significantly assoc w/ reduced growth, **β = –0.05, p<0.004** GA: ns effect of Cnb use (statistics not reported)	Included: SES Preg alcohol use Preg cocaine use Maternal age Pre-preg BMI Parity Diabetes Previous PTB Infant sex Father's height Alcohol*maternal age interaction Smoking* maternal age interaction Considered: Narcotics use	Strengths: Cited by authors: Numerous interviews lead to more precise measurement of substance use Ultrasound determination of GA Advanced statistical modelling Limitations: Substantial missing data for covariates: previous PTB was missing for 1037, diabetes missing for 770. Both variables were included in path analysis. Full information maximum likelihood estimation was employed to handle missing data Only included African-American women (92% of source population was African American) Authors state the quantification of drug use "can be somewhat confounded w/ no. of prenatal visits"

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Kliegman et al. 1994 Cleveland, OH	Prospective cohort Participants recruited from the University Hospitals of Cleveland and MacDonald Women's Hospital at time of delivery 1990 – 1991 N = ~4,000 women delivered at the hospital n = 425 "unselected" participants enrolled in anonymous urine screening protocol To avoid patient identification, infant GA and BW were recorded in categories Fisher Exact test, multivariate logistic regression	LBW PTB	Urine collected and screened at the time of delivery Preg drug history obtained by trained nurses, social workers, and staff of the maternity hospital's perinatal substance abuse program Self-report from interview, timing not specified	Participants who reported Cnb use or who had positive urine tests were categorized as Cnb users 34 (8.0%) women had a history of Cnb use or a positive urine test	<u>LBW:</u> OR = 2.28 (0.27, 19.5) <u>PTB:</u> OR = 1.89 (0.34, 10.50)	<u>Included:</u> Staff service (Medicaid) Black race Age <19 yrs Alcohol use during preg History of cocaine use Cocaine use at time of delivery Cigarette use No prenatal care Primiparous History of PTB <u>Considered:</u> Prior sexually transmitted disease Having >3 children	Sample selection not described; unclear what authors mean by "unselected". 53% of participants were Black. <u>Strengths:</u> Exposure assessed by self-report and urine test. <u>Limitations:</u> Small sample of Cnb users. No discussion or analyses of possible effects of co-use of multiple drugs. Focus of the study was cocaine use.

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Kline et al. 1987 New York City, NY	Prospective cohort 1975 - 1983 N = 3,783 women at 3 New York City hospitals who registered for prenatal care before 22 wks n = 2,735 women who delivered singleton, live births ≥28 wks w/ known BW Excluded: subsequent pregs of women already in the cohort, pregs w/ maternal heroin or methadone use Subjects were controls from a case-control study of SAB Multiple linear regression	BW adjusted for GA, determined by date of last menstrual period (LMP) reported by the woman	Interviews w/ women at average 20 wks gestation regarding drug and alcohol use, collected & analyzed in 2 overlapping phases Phase I (1975-1981) questionnaire only asked about drug use in the 3 mos prior to interview (n=1434) Authors state “for the most part, these are drugs used during pregnancy” Phase II (1979-1983) questionnaire asked about drug use 2 mos prior to LMP and during preg (n=1381) Phase I women less often report smoking Cnb ≥2/wk than Phase II women (2.8% vs 5.7%)	Frequency of Cnb use during preg (Phase I, Phase II, respectively): None (90.7%, 89.7%) <1/mo (4.7%, 2.9%) 2-4/mo (1.9%, 1.7%) 2-3/wk (1.3%, 2.1%) 4-6/wk (0.4%, 0.5%) Daily (1.0%, 3.1%)	Difference in BW (g) (SE) btwn Cnb users and nonusers by frequency of use, Phase I & Phase II, respectively: <1/mo: +36.9 (60.8), p>0.05 +30.4 (84.8), p>0.05 2-4/mo: **+199.1 (92.8), p<0.05** +6.6 (102.0), p>0.05 2-3/wk: −6.9 (112.0), p>0.05 −126.9 (96.9), p>0.05 4-6/wk: −222.5 (204.3), p>0.05 −143.3 (199.3), p>0.05 Daily: +84.9 (119.7), p>0.05 **−229.6 (79.3), p<0.01** Phase II showed a dose-response relationship of decreasing BW w/ increased frequency of Cnb use, but Phase I did not show a consistent assoc btwn BW and Cnb use	<u>Included</u>: Tobacco Sex of infant Gestation at delivery Gestation at interview Payment status (private vs public) Race Maternal birth place Pre-preg weight Parity Language <u>Considered</u>: Hospital Maternal age Education Previous SAB Previous LBW infant	Authors note that changes in Phase I & Phase II questionnaires may result in higher proportion of daily Cnb users. Authors speculate differences in observed effects of daily Cnb use btwn Phase I and Phase II may be due to changes over time in use of other illicit drugs, such as increasing cocaine use, possible changes in composition of Cnb, or contaminants or herbicide in Phase II. <u>Strengths</u>: Multiple Cnb exposure frequency categories Assoc w/ cigarette, alcohol, and Cnb exposure each analyzed <u>Limitations</u>: Frequency of Cnb use, but not amount used each time, was assessed

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Kline et al. 1991 New York City, NY	Case-control Participants from 3 hospitals in New York City 1979 - 1986 N = 2,853 cases N = 2,636 controls n = 960 cases of SAB <28 wks gestation where abortus could be retrieved and karyotyped n = 2,042 controls who registered for prenatal care in same hospitals and delivered after 28 wks, matched for cases' age (±2 yrs) Participants excluded after 1st study preg or for reporting other illicit substance use Conditional logistic regression, ANOVA	SAB of known karyotypes: Trisomy Monosomy X Triploidy Other chromosomally aberrant Chromosomally normal	One interview asking frequency (days/wk or mos or in total), in which mo, and for how long they first used Cnb and other drugs, during the period from 2 mos before LMP to the time of SAB or interview (controls) The perifertilization period was defined as 2 mos before LMP to 1 mo after LMP Women were not asked about daily Cnb intake	Analyzed Cnb use as both binary and continuous (mean days/wk) variable Cnb use dichotomized for analysis since including frequency of use added no new information, due to low rates of Cnb use There were 240 Cnb users and 2,762 non-users % Cnb users, mean (SD) days/ wk of use, by case status Chromosomally aberrant: 6.1%, 1.6 (2.3) Chromosomally normal: 9.3%, 1.9 (2.1) Controls: 8.0%, 2.4 (2.5) 42% of users reported use <1 day/wk, 44% reported use 1-6 days/wk, and 14% reported daily use	Assoc w/ Cnb use during perifertilization period comparing chromosomally abnormal SAB to chromosomally normal SAB (OR): Trisomy: 0.8 (0.4, 1.8) Monosomy X: 1.8 (0.7, 4.4) Triploidy: 1.3 (0.3, 4.6) Other aberrant: 1.2 (0.4, 3.6) Assoc btwn prenatal Cnb use at any time and SAB (OR): Chromosomally aberrant: 1.2 (0.7, 1.9) Chromosomally normal: 1.1 (0.7, 1.5)	Included: Maternal age Ethnicity Tobacco use Considered: Education Obstetric history Public assistance Hospital GA Alcohol Adjustment for alcohol or public assistance had little effect on the ORs, thus excluded in order to maintain statistical power	Unable to distinguish effects of use during preg from effects of use only in the perifertilization period, since nearly all users (97%) reported use both during and after the perifertilization period.

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Knight et al. 1994 Washington D.C., US Subjects drawn from a study that investigated the impact of nutrition and other outcomes of preg	Prospective cohort Participants were recruited from urban prenatal clinics n = 349 <u>Eligibility criteria:</u> Nulliparous No sickle cell anemia, thalassemia, hemoglobin C, or diabetes mellitus Student's *t*-test, Pearson's correlation coefficients, linear regression	BW BL HC GA	Maternal serum and cord blood samples analyzed w/ direct automated enzyme-mediated immune technique Random sample was selected for urine testing to validate blood samples Samples collected each trimester	Exposure was quantified per trimester based on Cnb concentration in maternal serum in ng/mL: 1) <25 ng/mL 2) 25-49 ng/L 3) 50-74 ng/mL 4) 75-99 ng/mL 5) ≥100 ng/mL 1st trimester (n=11) 2nd trimester (n=93) 3rd trimester (n=103)	Birth outcomes assoc w/ each concentration of Cnb (ng/mL), mean ± SE (during 3rd trimester) No significant assoc btwn growth outcomes and Cnb concentration <u>BW (g):</u> 1) 3246 ± 105 2) 3250 ± 086 3) 3226 ± 109 4) 3411 ± 154 5) 3543 ± 261 <u>BL (cm):</u> 1) 50.2 ± 0.6 2) 50.1 ± 0.4 3) 49.5 ± 0.5 4) 50.6 ± 0.6 5) 51.3 ± 0.6 <u>HC (cm):</u> 1) 35.0 ± 0.4 2) 34.2 ± 0.3 3) 34.6 ± 0.3 4) 35.3 ± 0.6 5) 34.9 ± 0.3 <u>GA (wks):</u> 1) 40.5 ± 0.5 2) 40.0 ± 0.3 3) 40.3 ± 0.3 4) 40.4 ± 0.2 5) 40.3 ± 0.3	No other variables were included in analysis Other licit substances were excluded from analysis because few participants had positive screenings for alcohol or tobacco	Participants were predominantly African-American. No instances of polydrug use were detected by biological assay. <u>Strengths:</u> Analyzed concentrations of Cnb.

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Leemaqz et al. 2016 Australia, New Zealand, Ireland, UK Data from the Screening for Pregnancy Endpoints (SCOPE) study	Prospective cohort Participants from hospital antenatal clinics, obstetricians, general practitioners, or community midwives invited to participate 2004 - 2011 N = 5,690 Study included nulliparous women w/ singleton preg Exclusion criteria: Conditions increasing risk for PE,SGA, PTB Cervical cone biopsy ≥3 terminations or miscarriages Major fetal anomaly or abnormal karyotype Interventions that might modify preg outcome n = 5,588 Chi-squared, Student's t test, Breslow-Day test, Maentel-Haenszel test, mixed effects logistic regression, linear mixed model	SGA (BW <10th percentile for maternal height, weight, parity, ethnicity, GA, and sex) Spontaneous PTB (SPTB; birth at <37 wks not resulting from medical or obstetric intervention) Preg complications (GHT, PE, GDM; not included in this table)	Participants examined and interviewed at 15±1 and 20±1 wks gestation Cnb use and cigarette smoking were identified by self-report of freq, no. of episodes of use, and no. of joints or cones used	No. of episodes of Cnb use for previous 3 mos was recorded as a continuous variable Cnb and cigarette exposure categories: Never used (n=5,273) 1) Quit prior to preg (n=95) 2) Quit prior to 15 wks gestation (n=145) 3) Still using at 15 wks, quit prior to 20 wks (n=22) 4) Still using at 20 wks (n=53) 5.6% of the sample reported Cnb use (range: 3.7% [UK]–11.6% [Australia])	OR (CI) for Cnb exposure categories, compared to never used Cnb: SPTB: 1) 2.23 (0.84, 5.86) p=0.106 2) 1.32 (0.55, 3.17) p=0.534 3) 2.76 (0.59, 13.01) p=0.199 4) **5.44 (2.44, 12.11) p<0.001** SGA: 1) 1.08 (0.50, 2.33) p=0.839 2) 0.95 (0.54, 1.65) p=0.843 3) 1.64 (0.56, 4.83) p=0.369 4) 1.84 (0.90, 3.76) p=0.095 OR (CI) for *any* Cnb use and preg outcomes, adjusted for *any* cigarette smoking, and smoking at 20 wks: SPTB: Adj for any smoking: **2.28 (1.49, 3.60) p<0.001** Adj for smoking >20 wks: **1.97 (1.29, 3.09) p=0.004** SGA: Adj for any smoking: 1.13 (0.80, 1.80) p=0.555 Adj for smoking > 20 wks: 1.04 (0.73, 1.47) p=0.917 Among women who continued Cnb use at 20 wks, 64% delivered at less than 32 wks gestation GA was lower for women who used Cnb >100 times in the 3	Included: Maternal age BMI SES Cigarette smoking Alcohol Interaction term for smoking*Cnb Recruiting center differences as a random effect	Data on no. of episodes of Cnb use were reported only in GA analysis. Data on other drug use in 3 mos prior to preg was recorded, but excluded due to insufficient data. Strengths: Used stratified analysis as well as interaction terms to compare risks assoc w/ Cnb among cigarette smokers and nonsmokers. Examined risks assoc w/ Cnb use in different exposure windows Limitations: Sample size for each outcome among Cnb users and use categories was relatively small (e.g., 11 cases of SPTB among women who reported Cnb use at 20 wks)

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					mos before 20 wks gestation, w/ estimated GA<37 wks for both cigarette smokers and non-smokers (p=0.002). The effect was weaker but still present among women who used Cnb at 15 wks (p=0.000) For all outcomes evaluated, risk assoc w/ Cnb use was independent of smoking status and SES (i.e., no interaction)		

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Linn et al. 1983 Boston, MA	Retrospective cohort Participants were women recruited from 84.4% of women delivering at the Brigham and Women's Hospital Boston Hospital for Women Division 1977 - 1980 N = 14,458 n = 12,424 mothers of singletons interviewed Reasons for not approaching women: insufficient personnel (random selection at these times), non-participating physician. Main reasons for no interview: early discharge, refusal. Exclusions: medical record not found, diabetic, record of "drug abuse" (due to use of other drugs) Medical records were reviewed for outcome data and malformations were classified w/ no knowledge of exposure status Logistic regression	LBW PTB SB BD PROM Placental abruption Fetal distress Apgar score	Women were interviewed during the delivery admission, after delivery Women were asked whether they used Cnb during preg, and if so, whether they used it "occasionally, weekly, or daily" w/ no further quantification or verification	1,246 women reported use of Cnb during preg: Occasional use 880 (7.1%) Weekly use 229 (1.8%) Daily use 137 (1.1%)	Cnb users vs. nonusers: LBW: OR = 1.07 (0.87, 1.31) p>0.05 PTB: OR = 1.02 (0.82, 1.27) p>0.05 SB: ORs not reported due to small nos. of SB Major malformations (OR = 1.36 (0.97, 1.91) Included congenital heart disease, hypospadias, clubfoot, upper alimentary tract, respiratory tract, genital, face/neck/ear, spina bifida, hydrocephalus, all others. Results for daily Cnb users were reportedly similar; data not reported Fetal distress, PROM, Apgar<6, premature labor, placental abruption: no adjusted analyses reported	Included Race Age Education Welfare status Smoking 3+ cigarettes/day at delivery 1st trimester alcohol use Parity Previous SBs Previous induced abortion Previous miscarriage PI (LBW analysis only) Alcohol use in preg Covariates were selected *a priori* and included in models. Cnb was considered predictive of an outcome if the full model provided more information than the model w/ only covariates	Greater percentage of women in sample had > high school education (65.68%) vs general population (49.03%). Authors note that their measurement of Cnb use was not explicit enough and that they did not collect any data on use by trimester.

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Mark et al. 2016 Baltimore, MD	Retrospective cohort 2009 - 2010 Chart review of all patients presenting for prenatal care at an urban, university- affiliated prenatal clinic N = 396 patients w/ adequate prenatal records n = 170 w/ delivery data (delivered at authors' institution) Demographic and behavioral characteristics, social work evaluation from prenatal charts Birth outcome data from hospital records, delivery summaries, and postpartum follow-up Chi-square, Fisher's exact test, ANOVA, logistic regression	BW LBW VLBW GA PTB NICU admission	Clinic universally screens women initiating prenatal care by written instrument and urine testing, after consent At intake, 29.8% of included patients screened positive through self-report or urine analysis, 54 patients screened positive by both urine and self-report Cnb screening questions (timing, frequency, amount, continuation in preg) were not reported Authors state continued use was monitored through urine testing each trimester and at delivery. No. of samples obtained in the 1st, 2nd, and 3rd trimesters were 160, 40, and 9, respectively	Primary exposure was any Cnb use, assessed by self-report or urine testing at first obstetrical visit	Unadjusted analyses except for LBW, VLBW <u>Mean BW</u> Cnb +ve: 3026 g Cnb –ve: 3089 g p=0.555 <u>LBW</u> OR = 0.87 (0.3, 2.54) <u>VLBW</u> OR = 5.87 (0.9, 38.4) <u>Mean GA</u> Cnb +ve: 38w 2d Cnb –ve: 38w 6d p=0.139 <u>PTB</u> Cnb +ve: 17.7% Cnb –ve: 12.0% p=0.325 <u>NICU admission</u> Cnb +ve: 25.5% Cnb –ve: 15.8% p=0.139 <u>Apgar < 8</u> At 1 min: Cnb +ve: 17.7% Cnb –ve: 14.4% p=0.592 At 5 min: Cnb +ve: 3.9% Cnb –ve: 4.2% p=1.000	<u>Included</u>: Age Race Marital status Education Employment Current tobacco use History of abuse <u>Considered:</u> Alcohol use	Most patients were African-American, unemployed, and had an unplanned preg. No measurable differences in Cnb use by availability of delivery data, i.e., no differential attrition. High prevalence of Cnb use may reflect local variation and/or universal screening w/ urine testing, as opposed to reliance on self-report alone. <u>Limitations</u>: Inconsistent urine testing after 1st trimester. Frequency, quantity, and duration of Cnb use in preg was not assessed. Possibility of exposure misclassification if women stopped using Cnb soon after learning they were preg but still tested +ve for Cnb by urine test.

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Massey et al. 2018 US	Meta-analysis n = 1,191 after exclusions and w/ complete data from 3 cohorts (2 prospective, 1 retrospective): Growing Up Healthy (GUH) cohort (2006-2009); n = 251 from N = 258 mothers ≥ 18 yrs, English-speaking, singleton pregs, recruited at ≤20 wks gestation, from an urban, hospital-based obstetric clinic Oversampled for tobacco exposure: Tobacco smokers were matched on age and education to closest eligible non-smoking woman Behavior & Mood in Babies and Mothers (BAM BAM) cohort (2006-2010 & 2012-2015); n = 315 w/ complete data from N = 353 mothers age 18 - 40 yrs, singleton pregs, recruited from obstetric clinics, community health centers, community postings Oversampled for tobacco exposure Early Growth & Development Study (EGDS) cohort (2003-2010);	BW LBW GA Sex of infant	Assessment of maternal substance use during preg followed Life History Calendar methods (EGDS) and Timeline Follow Back Interviews (GUH and BAM BAM). GUH and BAM BAM interviews were conducted during preg and at 3-6 mos postpartum. EGDS interviews were conducted at 3-6 mos postpartum GUH and BAM BAM used biomarkers to verify substance use in 47.5% of analytic sample. GUH assayed maternal saliva collected each trimester, and meconium collected at delivery and across several days after birth, in a single collection bottle for metabolites of Cnb, ethanol, amphetamines, opiates, and cocaine BAM BAM mothers provided saliva at 30 and 35 wks and at delivery for cotinine assays Infant meconium	Any Cnb use: n=273 (22.9%) Mean ± SD Cnb use among users (GUH and BAM BAM): 0.41 ± 0.73 joints/day Co-use tobacco & Cnb: n=230 (19.3%) Cnb exposure was included in models as binary variable Mean ± SD tobacco exposure among smokers: 6.15 ± 5.32 cigarettes /day	All substances used were moderately inter-correlated w/ one another, and inversely assoc w/ BW except alcohol use (no correlation) BW: Any Cnb use during preg, adjusted for tobacco use, was assoc w/ a ss decrease in BW **−84.37 g (−159.45, −9.28) p=0.028** change (reduction) in BW Any use of tobacco during preg was assoc w/ a ss decrease in BW, **−99.42 g (−166.94, −31.89) p=0.004** Cnb and tobacco co-use was assoc w/ a ns decrease in BW beyond individual effects of Cnb and tobacco (β=−55.15, (−127.10, 16.80)) GA: Neither Cnb use nor tobacco use was assoc w/ length of gestation Cnb (β = 0.14, (−0.13, 0.41)) p=0.30 There was no interaction effect of Cnb and tobacco on gestation length Sex of infant: Male infants had a **−153.09 g (−259.52, −46.66) p=0.005** ss decrease in BW assoc w/ any Cnb exposure and decrease in BW **11.00 g (-20.30, -1.70) (p=0.02)** ss	Included: Other drug use Tobacco use Alcohol use Maternal age at delivery Minority race/ ethnicity Education Male infant (unless sex-specific analysis) Interaction term for co-use of tobacco and Cnb	Authors intended to address the following, based on limitations of past studies: co-use of Cnb w/ tobacco, alcohol, and other drugs; quality of exposure measurement; power to detect effects; continuous rather than dichotomous measurement of outcomes; and potential sex differences in effects. Strengths: Examined outcomes both individually and combined for Cnb and tobacco use, as well as specific to gender of the infants. There was a high rate of Cnb use in the pooled cohort. Limitations Low prevalence of prenatal exposure to Cnb alone (n=25, 2.1%). Authors note that they did not utilize continuous measure of Cnb and other drug exposures, or account for timing of exposures in gestation. No mention of testing for heterogeneity.

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	n = 625 w/ complete data from N = 914 biological mothers and their infants born ≥28 wks and w/o major congenital conditions, placed w/ adoptive parents, recruited from adoption agencies in multiple regions Correlations, multiple linear regression		samples were collected for up to 3 days after delivery and assayed for metabolites of Cnb, cocaine, and other illicit drugs EGDS did not verify w/ biomarkers		assoc w/ each cigarette /day Female infants had an 8.26 g (CI −96.02, 112.55) p=0.88 change in BW ns assoc w/ any Cnb exposure and a -13.45 g decrease ns assoc w/ each cigarette/day Interaction terms for Cnb and tobacco co-use did not support an effect of co-use beyond the effects of each substance alone		

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Petrangelo et al. 2018 44 states in US	Retrospective cohort National Inpatient Sample from the US (database of ~7 million hospital admissions annually) Births cohort 1999-2013 N = 12,578,557 Participants were included if they were diagnosed w/ Cnb dependence or Cnb abuse Women not receiving this diagnosis constituted the reference group Multivariate logistic regression	IUGR (Fetal Growth Restriction) PTB SB (Intrauterine fetal demise) BD PE GDM Placenta previa PROM Chorio-amnionitis Mode of delivery Postpartum death Length of hospital stay	Diagnosis of Cnb abuse or dependence based on ICD-9 (using 304.3 and 305.2, identifying Cnb dependence or Cnb abuse)	Binary exposure quantification No Cnb abuse or dependence diagnosis (n=12,511,632) Cnb abuse or dependence diagnosis (n=66,925)	IUGR: OR = **1.35 (1.30, 1.41) p<0.0001** PTB: OR = **1.40 (1.36, 1.43) p<0.0001** SB: OR = **1.50 (1.39, 1.62) p<0.0001** BD: OR = 1.00 (0.88, 1.13) ns Preeclampsia: OR = **0.95 (0.91, 0.99) p<0.05** Gestational diabetes: OR = **0.76 (0.73, 0.80) p<0.001** Placenta previa: OR = **1.24 (1.11, 1.39) p<0.001** PROM: OR = **1.46 (1.35-1.58) p<0.001** Chorioamnionitis: OR = **1.18 (1.11, 1.25) p<0.001** Mode of delivery: Cesarean section: OR = **0.85 (0.84, 0.87) p<0.001** Forceps: OR = **0.82 (0.74, 0.91) p<0.00** Ventouse: OR = **0.81 (0.77, 0.85) p<0.001** Length of stay 3-6 days: OR = **0.97 (0.95, 0.99) p<0.001** Length of hospital stay >7 days: OR = **1.17 (1.11, 1.23) p<0.001** No significant assoc btwn Cnb use and postpartum death, postpartum hemorrhage, or	Included: Age Race Hospital location Insurance type Income Multiple births Hypertension Pre-existing diabetes mellitus Smoking Alcohol use Other illicit drug use	Authors note that women reporting Cnb abuse were more likely to be <25 yo, African-American, of the lowest 2 income quartiles, have Medicaid, and be admitted to an urban teaching hospital. They were also more likely to smoke, use alcohol, be hypertensive, or use other drugs during preg. The study did not clarify that the Cnb dependence or abuse diagnosis occurred during preg. Strengths: Large study population Limitations: Data was retrieved from a survey w/ substantial missing data, leading to high potential for residual confounding. Exposure information relied on self-report of the women and the assignment of diagnosis by health care professional. No quantification on timing or frequency of Cnb use.

Study/ Location	Study Design, Sample Sizes, Statistical Analysis	Outcomes of Interest	Exposure Measurement Methods	Exposure quantification	Results	Covariates/ Confounders	Comments
					venous thromboembolic disease		

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Quinlivan and Evans 2002 Three metro-politan hospitals in Australia	Prospective cohort Participants recruited from 3 antenatal hospitals in Australia 1998 - 2000 N = 503 eligible teenage antenatal patients <u>Inclusion criteria:</u> Women 12-17 yo Intended to continue preg Did not intend to relinquish their infant n = 456 Chi-squared, Fisher's exact test, Student's t test or Wilcoxon rank sum test, trend test across no drugs, Cnb, and multidrug groups	BW BL HC GA PTB PROM BW ratio: infant's BW divided by median BW for infant sex and GA, maternal parity and height Codable postnatal problems: prematurity, jaundice, febrile morbidity, weight loss, drug withdrawal Placental weight Apgar scores	Participants were interviewed in the antenatal period to establish drug use history before and during preg	93 mothers used Cnb throughout preg 31 of whom were multidrug users Participants were categorized as follows: Cnb only users (n = 62) Multidrug users (n = 93) No drug use (n = 363) Non-users were participants who never used Cnb, or ceased use before preg 50% of no drug use group reported ceasing drug use immediately prior to becoming preg	Threatened pre-term labor Cnb users versus no-drug use group: OR = **2.0 (1.1, 3.9)** HC: 0.4 cm reduction ns assoc w/ Cnb use test for trend p=0.08 There were ns assoc btwn Cnb use and BW, BW ratio, GA, PTB or other growth outcomes No significant assoc w/ PROM or any codable postnatal problems	<u>Included:</u> Pre-preg weight Maternal height age and race GA Gender Significant antenatal assoc of illegal drug use Smoking Alcohol	Authors note that those who participated in the study may have had superior prenatal care, improving birth outcomes. Social isolation, homelessness, and domestic violence were significantly more common in the Cnb and multidrug use groups. Psychiatric diagnosis were also significantly higher in the drug use groups. Authors note that though indigenous women represent a high proportion of teenage preg, continued illegal drug use was similar btwn races. Participants who used illegal drugs in preg weighed significantly less than the no-drug use group. <u>Strengths:</u> There was a Cnb only group. A subgroup of 180 mothers were interviewed at 6-mos postpartum. There was a high degree of agreement (94.9%) for answers about drug use btwn antenatal interviews and the 6-mo interview.

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Saurel-Cubizolles et al. 2014 France	Retrospective cross-sectional study N = 14,681 A nationally representative sample of births Eligibility criteria: Delivered in France during 1 wk in March 2010, w/ GA >22 wks or BW ≥ 500g at birth n = 13,545 who had live-born singletons and answered questions about Cnb use Chi-squared, multivariate logistic analysis, generalized linear regression	PTB: Medically indicated PTB (MIPTB) Spontaneous PTB (SPTB) SGA (binary) BW (continuous) GA (categorical, not a primary outcome)	Participants were interviewed 2-3 days postpartum Participants were asked if they ever used Cnb, and if so how often	No use (n=13,531) Less than once/mo (n=93) Once/mo or more (n=65)	Prevalence of cnb use during preg: 1.2% Cnb use <1/mo vs ≥1/mo Outcomes for total sample: PTB OR (95% CI): <1/mo: 1.62 (0.78, 3.40) ≥1/mo: **2.22 (1.04, 4.74)** SPTB OR (95% CI): <1/mo: 1.85 (0.75, 4.64) ≥1/mo: **2.57 (1.01, 6.59)** MIPTB OR (95% CI): <1/mo: 1.28 (0.40, 4.12) ≥1/mo: 1.62 (0.50, 5.27) SGA OR (95% CI): <1/mo: 1.51 (0.84, 2.71) ≥1/mo: **1.98 (1.07, 3.68)** BW (g): no use: 3303 <1/mo: 3157 ≥1/mo: 3054 Trend test **(p<0.001)** Among tobacco smokers: PTB OR (95% CI): <1/mo: 1.86 (0.64, 5.44) **≥1/mo: 2.68 (1.16, 6.20)** SPTB OR (95% CI): <1/mo: 1.63 (0.37, 7.23) **≥1/mo: 3.50 (1.28, 9.53)** MIPTB OR (95% CI): <1/mo: 2.06 (0.47, 9.08) ≥1/mo: 1.49 (0.34, 6.41) SGA OR (95% CI): <1/mo: 1.29 (0.61, 2.72) ≥1/mo: 1.98 (0.66, 2.56)	Included: Maternal age Parity Nationality Cohabitating Education level Employment Household income BMI Alcohol consumption No. of cigarettes/ day in 3rd trimester (for smokers only) GA (for BW analysis)	Cnb use was more common among women who were younger, living alone, or who had a low level of education or income. Low prevalence of reported use (recreational Cnb use is illegal in France). Strengths: Source population was a nationally representative sample of births. Stratified by tobacco smoking status. Analyses included frequency of use and test for trend. Limitations: Strong potential of under-reporting, so assoc were probably underestimated. Evaluated Cnb use postpartum.

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					BW (g): no use: 3151 <1/mo: 3016 ≥1/mo: 3010 Trend test **p<0.01)** Among non-tobacco smokers PTB OR (95% CI): <1/mo: 1.24 (0.44, 3.49) SPTB OR (95% CI): <1/mo: 1.22 (0.29, 5.06) MIPTB OR (95% CI): <1/mo: 1.22 (0.29, 5.11) SGA OR (95% CI): <1/mo: 1.24 (0.52, 2.94) BW (g): no use: 3335 use: 3244 (only 12 women did not smoke tobacco and used Cnb, thus stratification on frequency was not possible Percentage of births before 32 wks was higher among Cnb users Tobacco-Cnb interaction was ns for any preg outcome		

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Schempf and Strobino 2008 Baltimore, MD	Retrospective cohort Participants in original study were low-income women, ≥19 yrs old, who delivered at one hospital in 1995 - 1996 N = 1,201 All women w/ evidence of drug use (medical record, self-report, or urine screen) or no prenatal care, and 2/3 of remaining women were selected n = 808 participants who delivered live, singleton births Birth and medical data obtained from medical records Chi-squared test, linear and logistic regression	BW LBW	Cnb and other drug use were determined by self-report at screening or any of the following: Postpartum in-person interview to obtain detailed drug use history and information on social and psychosocial factors Universal urine screen for Cnb, cocaine, and opiates performed at admission to labor and delivery Participants' medical records (screened for medical history and drug use history)	Exposure quantification was binary. 15% of participants were categorized as Cnb users based on positive results from self-report, urine screen, or medical record Postpartum interview ascertained monthly, weekly, or daily use of Cnb	Results reported as unadjusted and adjusted for multiple sets of risk factors (not all included here). All adjusted results are ns. BW Adjusted for other drug use β=–0.2 (–140.6, 140.2) g Adjusted for social, psychosocial, behavioral, and biomedical factors β=–24.6 (–155.8, 106.5) g LBW: Adjusted for other drug use OR=0.93 (0.55, 1.57) Adjusted for social, psychosocial, behavioral, and biomedical factors OR=1.07 (0.60, 1.92)	Included: Alcohol Other drug use Tobacco Social factors: maternal age, money for necessities, housing Psychosocial factors: stress, preg locus of control Behavioral factors: early prenatal care Biomedical factors: hypertensive disorders Other medical risk factors: pre-preg weight, net weight gain Many other variables examined in bivariate analyses	The majority of the sample was Black and only 12% had education beyond high school Authors state some biomedical variables may be the direct effect of drug use; thus, adjustment for these variables may have introduced bias Authors state the results suggest that illicit drug use is a stronger risk marker than a risk factor for adverse birth outcomes Strengths: Adjusted for a variety of social, psychosocial, behavioral, and biomedical variables Multiple methods for exposure assessment Limitations: GA not considered

Study/ Location	*Study Design, Sample Sizes, Statistical Analysis*	*Outcomes of Interest*	*Exposure Measurement Methods*	*Exposure quantification*	*Results*	*Covariates/ Confounders*	*Comments*
Shankaran et al. 2004 RI, FL TN, MI	Retrospective cohort Women were recruited after delivery in one of 4 study centers All mothers of term (≥38 wks) infants <1500g, and all mothers of term infants >1500 g during defined recruitment hours were eligible <u>Exclusion criteria</u> Birth outside the catchment area for follow-up Age <18 yrs Multiple birth Maternal psychosis N = 801 Maternal and infant data were obtained through interviews, medical charts, and meconium analysis for metabolites of illicit drugs At 1 mo of age, 2 infant groups were selected: 1) exposed to cocaine or opiates in utero 2) not exposed to cocaine or opiates, matched to each exposed infant by race, gender, and GA at each site. n = 651	BW BL HC	All infants' meconium analyzed w/ enzyme multiplied immunoassay technique, and GC-MS of all +ve samples Maternal Inventory of Substance Use (MISU) administered by trained interviewers at 1-month follow-up to assess use of cocaine, opiates, Cnb, alcohol, and tobacco during 4 time periods: 3 mos before preg, each trimester Early period: 3 mos before preg and 1st trimester Late period: last 2 trimesters Use during either period was based on maximum quantity reported	Participants w/ any admitted use and positive meconium samples were considered Cnb exposed (n=205) <u>Use frequency categories</u>: High: 1 to ≥3 joints more often than 1 -2 days/wk, or <1 joint every day Moderate: >1 joint 1 -3 days/mo or <1 joint 1-2 days/ wk Low: <1 joint 1-3 days/mo <u>Patterns of use</u>: Consistently high Increasing use (increasing from the early to the late period) Consistently moderate Decreasing use (decreasing from the early to the late period) Consistently low	Cnb use was not significantly assoc w/ any outcome Data presented as a forest plot w/ no numerical measures of assoc <u>BW:</u> High Cnb use assoc w/ ns decrease in BW Moderate and low Cnb use assoc w/ ns increase in BW Decreasing Cnb assoc w/ ns increase in BW <u>BL:</u> High and moderate Cnb use assoc w/ no change Low and decreasing Cnb use assoc w/ ns increase <u>HC:</u> High Cnb use assoc w/ ns decrease in HC Moderate, low, and decreasing Cnb use assoc w/ ns increase in HC	<u>Included:</u> Patterns of use of cocaine, tobacco, alcohol Clinical site Maternal race Maternal age Parity Pre-preg weight GA Infant gender SES	Excluded participants tended to be older and had higher incidence of cocaine use 90 women who denied drug use but had positive meconium results, and 4 women who reported cocaine use, but had negative meconium results were excluded <u>Strengths:</u> Examined effects of various patterns of drug use Evaluated only full term infants, allowing for more accurate growth assessment <u>Limitations</u> Heavy focus on cocaine, less information about Cnb High frequency of multi-drug use among participants which obscures results for individual drugs Relied on self-report 1 mo after birth for behaviors that occurred throughout and before preg Small Cnb only group (n=3). All other Cnb-exposed were also exposed to some combination of cocaine,

Study/ Location	Study Design, Sample Sizes, Statistical Analysis	Outcomes of Interest	Exposure Measurement Methods	Exposure quantification	Results	Covariates/ Confounders	Comments
	Chi-squared test, multivariate linear regression, Tukey-Kramer test for multiple comparisons						alcohol, and tobacco. 141 women had used no drugs.

Study/ Location	*Study Design, Sample Sizes, Statistical Analysis*	*Outcomes of Interest*	*Exposure Measurement Methods*	*Exposure quantification*	*Results*	*Covariates/ Confounders*	*Comments*
Shiono et al. 1995 Multi-center Oklahoma City, OK, New York City, NY, New Orleans, LA, San Antonio, TX, Seattle, WA Study participants were selected from the Vaginal Infections and Prematurity Study	Prospective cohort Participants were women who received prenatal care in clinical centers 1984 – 1989 N = 13,914 <u>Eligibility criteria:</u> Intend to deliver at a study hospital Single gestation Not having any of the following: Diabetes requiring insulin Hypertension or heart disease requiring medication Chronic renal disease Rh sensitization Current use of corticosteroids History of cervical incompetence or cerclage Antibiotics in past 2 wks Use of tocolytic agents 10,695 provided blood samples, 3,225 had insufficient sera available n = 7,470 Chi-squared test performed for bivariate analysis, multivariable logistic regression performed for statistically significant variables. Mantel-Haenszel estimate used to calculate odds	LBW PTB Placental abruption	Self-report from interview at 23-26 wks gestation Serum samples were obtained at 23-26 wks and again at 31-36 wks. Women who declared use of illicit drugs were asked about weekly frequency of use Serum assay for Cnb and cocaine, analyzed by radioimmunoassay	Exposure quantification was binary. If a participant reported Cnb use or had a positive serum sample, they were categorized as Cnb users Cnb users (n = 822) Non-users (n = 6,476)	Cnb users defined by self-report <u>or</u> serum assay at 23-26 wks <u>LBW:</u> Cnb users w/ LBW infants compared to non-users Cnb +ve: 11% Cnb –ve: 7.7% **p<0.01** OR = 1.1 (0.9, 1.5) <u>PTB</u>: % of Cnb users w/ PTB infants compared to non-users Cnb +ve: 13.4% Cnb –ve: 11.8% **p<0.05** OR = 1.1 (0.8, 1.3) <u>Placental abruption:</u> OR = 1.3 (0.6, 2.8) Cnb use based <u>only on serum assay</u> LBW: OR = 1.5 (1.2, 2.0) PTB: OR = 1.3 (1.0, 1.7) Cnb use based <u>only on self-report</u> LBW: OR = 1.0 (0.7, 1.4) PTB: OR = 1.1 (0.8, 1.6) A ss assoc was observed btwn cigarette smoking and LBW	<u>Included:</u> LBW in previous preg Study institution Ethnicity BMI Trhichomonas vaginalis infection Tobacco smoking Alcohol Cocaine	Primary reason for serum collection was to detect acute infections, thus women who did not have sufficient serum volume for drug assays were not included in the study 43% of women were African-American, 33% were white or other. Study compared self-report results to serum results. Cnb was detected in serum in 585 (7.8%) of women Cnb use was reported by 417 (5.6%). Overall 822 (11%) of women reported use <u>or</u> had positive serum samples. Overall 180 women (2.4%) reported use <u>and</u> had a positive assay. Of the 585 who tested positive for Cnb, 180 (~31%) also reported Cnb use. Whereas, of the 417 women who reported Cnb, 180 (~43%) also had a positive serum assay. Authors note that individuals identified as users by serum samples are more likely to be

Study/ Location	Study Design, Sample Sizes, Statistical Analysis	Outcomes of Interest	Exposure Measurement Methods	Exposure quantification	Results	Covariates/ Confounders	Comments
	ratio adjusted for smoking in cases w/ rare outcomes						heavy-users, as the window for detection by biological assay is small Strengths: Two methods of exposure screening were used. Authors note that population based-screenings that rely on biochemical markers alone consistently underestimate prevalence of prenatal drug exposure Limitations: Did not analyze the quantity of Cnb used during preg.

Study/ Location	*Study Design, Sample Sizes, Statistical Analysis*	*Outcomes of Interest*	*Exposure Measurement Methods*	*Exposure quantification*	*Results*	*Covariates/ Confounders*	*Comments*
Tennes et al. 1985 Denver, CO	Prospective cohort 1981 - 1982 N = 1,032 women, ≥24 wks preg, predominantly lower-middle to lower SES, approached at prenatal clinics of Denver General and University Hospitals n = 756 infants of mothers who completed 2 interviews Exclusions: Non-English speaking Diabetes Renal or collagen disease Structured interview for information on demographics, illnesses, medications, use of caffeine and other substances, and drug use by child's father 24-72 hours after delivery, 2nd interview to ascertain drug use since 1st interview, and infants were assessed Infants of selected Cnb users and nonusers examined at 1 yr and mothers interviewed Chi square and multivariate analyses	BW BL HC PI GA PTB BD Delivery complications Brazelton Neonatal Behavioral Assessment Scale (BNBAS) Muscle tone Weight, height, Mental Score, Motor Score at 1 yr Sex ratio	Cnb information collected from interviews before and after birth No. of times drug used/wk and no. of joints weekly summed to estimate drug exposure for entire preg or by trimesters	Total amount of Cnb (no. of joints) used during preg Categories of Cnb use (proportion of women in each category for trimester 1, 2, 3): Nonusers (68%, 79%, 84%) Light: one time only to once/wk) (12%, 10%, 9%) Moderate: > once/wk wk, less than daily: (10%, 6%, 5%) Heavy: ≥ once daily (10%, 4%, 2%) Other illicit drugs measured as no. of occasions of use	BW, HC not assoc w/ Cnb use (no data presented) BL reduced 0.07 cm in 1st trimester Cnb users compared to nonusers (**p=0.001**), but n.s. in 2nd and 3rd trimester users, and for total Cnb smoked. Smoking 3 joints/day in 1st trimester was assoc w/ an unadjusted **0.55 cm** reduction in BL GA positively assoc w/ total Cnb used in preg (β = **0.09, p=0.03**) PTB, BD, muscle tone, Apgar score<7, and BNBAS (including cluster scores) not assoc w/ Cnb Delivery complications: Cnb use was assoc only w/ need for increased pain medication during labor, which authors suggest may be due to primiparity among Cnb users An analysis of 1-yr-olds of randomly selected heavy and moderate Cnb users and non-users found no significant differences in weight, height, mental score, motor score Cnb was not assoc w/ infant temperament, mother's report of illnesses, eating, or sleeping problems, or personality characteristics in 1st yr	Included in analyses, indicated by superscript: Mother's height[1] Parity[3] GA[1] Infant sex[1,3] Nicotine[1] Weight gain[1,2] Ponderal index[1,2] Black race[1,2] Preg complications[2] 1=BL 2=GA 3="infant development" (unclear which outcomes) Considered in BW, BL, HC analyses: Mother's age Hispanic race SES Abortion Caffeine Alcohol Hash Amphetamines Cocaine	Cnb assoc w/ alcohol but not nicotine use. Daily users decreased mean Cnb use from 4.5 joints/day in 1st trimester to 1 joint/day in the 3rd trimester. Cnb users significantly differed from nonusers in age, marital status, no. of prior births and induced abortions, and gained more weight during preg. Strengths: 2 investigators were present at all examinations and blind to exposure, and BNBAS scales were scored independently by each.

Study/ Location	Study Design, Sample Sizes, Statistical Analysis	Outcomes of Interest	Exposure Measurement Methods	Exposure quantification	Results	Covariates/ Confounders	Comments
					Total Cnb use in preg, controlling for sex and parity, not assoc w/ “growth or development scores” in 1-yr olds Sex ratio: heavy maternal Cnb and paternal use were assoc w/ 61% and 67% male infants, respectively (**p=0.004 for fathers;** not reported for mothers)		

Human Studies: Birth Outcomes

Study/ Location	*Study Design, Sample Sizes, Statistical Analysis*	*Outcomes of Interest*	*Exposure Measurement Methods*	*Exposure quantification*	*Results*	*Covariates/ Confounders*	*Comments*
Thompson et al. 1994 New Zealand	Case-control Sample was randomly selected from the control group of the New Zealand case-control cot death study N = 1,800 Obstetrical records examined for 1762 participants Interviews conducted for 1592 participants Excluded: 85 were <37 wks gestation n = 157 SGA n = 1519 full-term not SGA Unconditional logistic regression Population attributable risk	SGA	Prenatal interview completed after birth Mean age for interview was 16.9 wks	Participants who reported any Cnb use during preg were categorized as Cnb users n=95 No further quantification	<u>SGA:</u> OR = 1.86 (0.98, 3.52)	<u>Included</u> Cigarette smoking SES Maternal education Maternal age Parity Racial/ethnic group Antenatal care in first 3 mos Marital status <u>Considered</u> Social support Parity Antenatal classes Urinary tract infection Demographic variables Caffeine Alcohol	Authors noted “although the prevalence of using marijuana during pregnancy was low, we found the use of marijuana was associated [with] small for gestational age at the 6% level after controlling for potential confounders including tobacco smoking.” <u>Limitations:</u> Prevalence of Cnb use during preg was low.

Study/ Location	*Study Design, Sample Sizes, Statistical Analysis*	*Outcomes of Interest*	*Exposure Measurement Methods*	*Exposure quantification*	*Results*	*Covariates/ Confounders*	*Comments*
van Gelder et al. 2009 AR, CA, GA, IA, ME, NJ, NY, NC, TX, UT The National Birth Defects Prevention Study	Case-control n = 10,241 cases and n = 4,967 controls, born 1997 - 2003 Cases had major congenital malformations (BD), identified from state birth defects surveillance systems and reviewed by clinical geneticists Controls were liveborn infants w/out major BDs, randomly selected from birth certificates or hospital records from the same regions. Excluded mothers w/ preexisting diabetes (type 1 or 2) Mothers were interviewed by phone, 6 wks to 24 mos after delivery using a standardized questionnaire to collect info on drug use, demographics, lifestyle, maternal health, occupational exposures Logistic regression	BD w/ at least 250 cases (20 types) and completed maternal interview included: Anencephaly craniorachiscchisis Spina bifida Anotia, microtia Dextrotrans-position of the great arteries Tetralogy of Fallot Hypoplastic left heart syndrome Coarctation of aorta Pulmonary valve stenosis Perimem-branous ventricular septal defect (VSD) Atrial septal defect	Information on type, timing, and frequency of maternal illicit drug use obtained from interviews Cnb (including medical use) and hashish were included in the Cnb drug group Infant defined as *exposed* if mother reported use of Cnb at any time during periconceptional period (1 mo before preg to end of 3rd mo of preg), and *unexposed* if mother reported no use of any illicit drug in the 3 mos prior to and throughout the entire preg	Periconceptional Cnb use was quantified as frequency per wk or day, and grouped as follows: No use Incidental use (≤ 1 time/wk) Moderate use (> 1 time/wk, but < 1 time/d) Heavy use (≥ 1 time/day) 420 (4%) cases and 190 (4%) controls exposed to Cnb	ORs were calculated for a drug category if there were ≥3 exposed cases If an assoc was found, exposure time window was limited to etiologically relevant period for that specific BD Anencephaly OR = **2.5 (1.3, 4.9)** for Cnb use in 1st mo of preg; Cnb use in other mos of periconceptional period was not assoc w/ anencephaly No pattern of increasing or decreasing ORs for the selected BDs was detected after stratification for frequency of peri-conceptional Cnb use, no substantial differences in crude ORs btwn women who used only Cnb and women who used Cnb and other drugs (data not provided) No BDs were assoc w/ Cnb use in the adjusted, 4-mo exposure model Gastroschisis was assoc w/ Cnb in unadjusted model, OR = **3.6 (2.7, 4.9)** After adjustment for maternal age, OR = 1.3 (0.9, 1.8)	Included: Maternal age at delivery Maternal race/ ethnicity Level of education Cigarette smoking in periconceptional period Binge drinking (≥ 4 drinks/ episode) in peri-conceptional period Prepreg BMI Periconceptional folic acid use	Strengths: Participation rates and maternal characteristics were comparable for cases and controls. Limitations: Multiple comparisons

Study/ Location	Study Design, Sample Sizes, Statistical Analysis	Outcomes of Interest	Exposure Measurement Methods	Exposure quantification	Results	Covariates/ Confounders	Comments
		(ASD) secundum					
		ASD not otherwise specified					
		Cleft lip ± cleft palate					
		Cleft palate					
		Esophageal atresia ± trachea -esophageal fistula					
		Anorectal atresia					
		Hypospadias					
		Transverse limb deficiency					
		Cranio-synostosis					
		Diaphrag-matic hernia					
		Gastro -schisis					

Study/ Location	*Study Design, Sample Sizes, Statistical Analysis*	*Outcomes of Interest*	*Exposure Measurement Methods*	*Exposure quantification*	*Results*	*Covariates/ Confounders*	*Comments*
van Gelder et al. 2010 Data from the US National Birth Defects Prevention Study (NBDPS), a case-control study on prenatal causes of BD	Retrospective cohort from a case-control study N = 5,871 <u>Eligibility criteria:</u> Completed NBDPS survey btwn 1997 and 2004 Available birth outcome data from hospital records or birth certificates Accurate birth records No missing birth data Singleton birth 16 infants excluded for incorrect records, 20 for missing data, and 174 w/ multiple gestations (more than one fetus) n = 5,661 n = 189 participants who reported use of Cnb during preg Multivariable linear regression BW and GA Multivariable logistic regression LBW and PTB In certain models data were stratified by trimester of use	BW LBW GA PTB	Women were surveyed via telephone about illicit drug use 6 wks - 2 yrs after delivery (self-reporting) Interviews reviewed maternal demographics, health, and included questions about the type, timing, and frequency of drug use from 3 mos before preg until birth Data on BW and GA were obtained through abstraction of birth hospital records or birth certificates	Drug use from 3 mos before preg until birth Type, timing, and frequency of drug use were asked 5 types of drugs: Cnb, cocaine, stimulants, hallucinogens, and opioids	Cnb use was ns assoc w/ mean BW, GA, LBW or PTB <u>BW (g)</u>: β (95% CI) Any Cnb during preg: Overall: -17 < (–90, 56) Non-smokers: -31 (-164, 101) Smokers: -14 (-102, 75) <u>LBW:</u> OR (95% CI) Any Cnb during preg: Overall: 0.7 (0.3, 1.6) Smokers: 0.7 (0.3, 2.0) <u>GA (wks):</u> β (95% CI) Any Cnb during preg: Overall: -0.1 (-0.4, 0.3) Non-smokers: 0.2 (-0.3, 0.7) Smokers: -0.2 (-0.6, 0.3) <u>PTB:</u> OR (95% CI) Any Cnb during preg: Overall: 1.0 (0.6, 1.9) Non-smokers: 0.6 (0.1, 1.9) Smokers: 1.2 (0.7, 2.1) Stratification by trimester of Cnb use (and smoking) did not yield any ss assoc	<u>Included</u> GA (for BW and LBW) Maternal age at delivery Race/ethnicity Cigarette smoking Binge drinking (≥4 drinks per sitting) Pre-preg BMI Gestational weight gain <u>Considered</u> Level of education Employment status Parity Previous induced abortions Periconceptional folic acid use Paternal drug use	Cnb users were more often non-Hispanic Black, had lower levels of education, a household income below $20,000 or unemployed, more likely to be underweight, or have excessive weight gain during preg. Cnb users were less likely to use folic acid in periconception period, and more likely to use alcohol and to smoke. <u>Strengths:</u> Stratified use by trimester and cigarette smoking. <u>Limitations:</u> Selected from a control cohort of NBDPS (births w/ defects have been excluded), who were relatively healthier w/ lower rates of LBW and PTB compared to the US general population. Very few cases in the exposed group may limit the statistical power to identify effect, especially in stratified analysis. Data from a telephone survey, great potential of under-reporting, subject to misclassification bias.

Study/ Location	*Study Design, Sample Sizes, Statistical Analysis*	*Outcomes of Interest*	*Exposure Measurement Methods*	*Exposure quantification*	*Results*	*Covariates/ Confounders*	*Comments*
Williams et al. 1991 Boston, MA	Nested case-control 1977 - 1980 N = 12,825 women w/ singleton deliveries, interviewed during postpartum hospital stay, from a cohort study of birth outcomes assoc w/ prior induced abortion n = 143 placental abruption cases n = 1,257 controls randomly selected from noncases at 9:1 control:case ratio Exclusion criteria: early discharge, refusal, language barrier, precluding medical conditions Logistic regression	Placental abruption, from medical records	Interviews w/in 1 or 2 days after delivery by trained interviewers who were unaware of study hypotheses, using a standard questionnaire No other information on assessment of Cnb use was reported	Cnb use during preg (cases, controls): At least weekly (n=9, 28) Occasional (n=13, 84) None (n=121, 1145)	At least weekly Cnb use was ss assoc w/ placental abruption: OR = **2.8 (1.2, 6.6)** Occasional use: OR = 1.4 (0.8, 2.6); OR not reported A positive trend was found btwn frequency of Cnb use and risk for placental abruption, **p=0.003** Interaction of covariates was considered, but ns Cigarette smoking was marginally assoc w/ placental abruption: adjusted OR = 1.5 (1.0, 2.2)	<u>Included</u>: Prior SB Chronic hypertension Maternal age ≥35 Pre-preg BMI<18 Prior SAB Tobacco Cervical incompetence Prior induced abortion Preg-induced hypertension Alcohol in 1st trimester Parity Race Medicaid payment status In utero exposure to diethylstil-bestrol Non-gestational diabetes Coffee during 1st trimester Later prenatal care registration Considered variables were not explicitly stated	Authors note the results are consistent w/ previous hypothesis that hypoxemia resulting from uterine dysfunction assoc w/ maternal aging, undernutrition, cigarette and Cnb smoking, and chronic hypertension may cause increases in uterine arterial pressure and lead to placental abruption.

Study/ Location	*Study Design, Sample Sizes, Statistical Analysis*	*Outcomes of Interest*	*Exposure Measurement Methods*	*Exposure quantification*	*Results*	*Covariates/ Confounders*	*Comments*
Williams et al. 2004 Atlanta, GA	Atlanta Birth Defects Case-Control Study n = 3,151 (122 cases, 3,029 controls, born 1968 - 1980) Cases were live and stillborn infants identified through the Metropolitan Atlanta Congenital Defects Program (MACDP) active surveillance system Of 615 cases w/ any kind of ventricular septal defect (VSD), 413 (67%) were interviewed and 122 met the case definition of isolated simple VSD for this study Infants diagnosed after 1st yr of life were not included Controls were randomly selected from all live births w/out birth defects in the same area and period, and frequency matched to cases by race, birth period, and hospital. 71% of selected controls participated Mantel-Haenszel test; unconditional logistic regression	Isolated simple VSD was defined as VSD alone or w/ an atrial septal defect or patent ductus arteriosus Isolated complex, multiple, and syndromic VSD were excluded The type of VSD was classified in part by the MACDP's detailed clinical information about each infant's assoc cardiac and noncardiac defects	Two-part phone interview conducted w/ both mother and father of infant 1st part asked for mother's reproductive history; this interviewer was not blinded to case status 2nd part ascertained periconceptional exposures (from 3 mos prior to preg through 1st trimester), including alcohol, cigarette, and illicit drug use Parents were asked to report their own and the other parent's exposures Mothers and fathers were interviewed by separate interviewers who were blinded to case status Cnb was combined w/ hashish use	Frequency of periconceptional Cnb use Categories (n based on maternal self-report): No use (n=102 cases, 2791 controls) Light use (≤2 days/wk; n=14 cases, 174 controls) Heavy use (≥3 days/wk; n=6 cases, 44 controls)	Maternal Cnb use was more prevalent among cases according to maternal self-report (p<0.01) and paternal proxy-report (p=0.02) OR (ordinal) for VSD and maternal Cnb use: **1.90 (1.29, 2.81)** The OR represents the excess risk assoc w/ light Cnb use vs no use, and heavy Cnb use vs light use OR for mothers who reported heavy alcohol and Cnb use was **7.51 (2.40, 23.55**). OR for maternal heavy alcohol use w/out Cnb was ns Crude ORs for VSD and maternal Cnb use according to paternal proxy-report: Any Cnb use **2.21 (1.11, 4.38**) Light use 1.54 (0.61, 3.89) Heavy use 3.19 (0.61, 10.71) Elevated unadjusted ORs for maternal Cnb use were maintained among women who reported no other illicit drug use	<u>Included:</u> Maternal age Multivitamin use (during periconceptional period vs no use or use outside periconceptional period) Maternal overt diabetes Infant race (White vs non-White) <u>Considered:</u> Maternal heavy alcohol consumption (not assoc w/ VSD in multivariate analyses) Cigarette use (not assoc w/ VSD) Use of cocaine, heroin, hallucinogens, and methadone was not included in analyses due to low prevalence of exposure (<2% of cases and <1% of controls)	The authors used paternal proxy report to combat underreporting of exposure due to social stigma; however, fathers reported lower prevalence of maternal Cnb use. Compared w/ control infants, case infants were more likely to be nonwhite, to be born to mothers younger than 20 yrs of age, and to have mothers w/ overt diabetes. Authors noted the study's findings might not apply to asymptomatic cases diagnosed after the child's 1st birthday.

Study/ Location	*Study Design, Sample Sizes, Statistical Analysis*	*Outcomes of Interest*	*Exposure Measurement Methods*	*Exposure quantification*	*Results*	*Covariates/ Confounders*	*Comments*
Zuckerman et al. 1989 Boston, MA	Prospective cohort Boston City Hospital prenatal clinics 1984 - 1987 N = 1,932 eligible Reasons for exclusion: refused, left clinic before interview, lost to follow up, delivered elsewhere, elective abortion, discharged before final interview, refused follow-up interview, delivered after end of study period, infant was not examined 17 participants had SAB, SB or had infants who died soon after birth n = 1,226 Chi-square, t test, multiple linear and logistic regression	BW BL HC GA Congenital anomalies Apgar scores	Self-report by prenatal and postnatal interviews about the timing and frequency of prenatal Cnb, alcohol, cocaine, tobacco, and other drug use First interview discussed patterns of drug use from 3 mo pre-preg to the date of interview; Second interview, postpartum, discussed drug use for the remainder of preg Urine samples were collected at both interviews and assayed for Cnb and cocaine metabolites Urine samples were screened using the enzyme-mediated immunoassay technique w/ cutoff for positive definition at 0.02 mg per liter. Samples that tested positive were retested using GC-MS	Positive by urine assay (n = 202) Positive by self-report, negative by urine assay (n = 129) Negative by self-report and urine assay (n = 895) Positive by self-report only and positive by urine assay were treated as separate variables for multivariate analysis	Of the 202 women w/ positive urine samples, 53 self-reported no use Multiple regression analysis for a positive urine assay: BW = **-79 g (p=0.04)** BL = **-0.52 cm (p=0.02)** HC = -0.19 cm (p=0.15) There were no significant findings for self-reported Cnb use and BW, BL, HC No interaction was observed btwn Cnb use and cocaine use for BW, BL, HC, and GA in the multiple regression analyses Univariate analysis: Prenatal Cnb use was ns assoc w/ GA or congenital anomalies (defined as ≥3 minor or 1 major anomaly) (there were 9 anomalies in the Cnb nonuse group, n = 895, and 9 in the Cnb use group, n = 202)	Included: Cocaine use GA Pre-preg weight Ethnicity Parity Prenatal care Tobacco Considered: Maternal age Maternal weight gain STDs during preg	Population was young, low income, primarily Black (66%) or Hispanic (18%) women. 16% of women who used Cnb were identified by urine tests alone and would have been misclassified as nonusers w/out urine testing. Authors noted that in studies relying on self-report only, there may be misclassification of Cnb users as nonusers, resulting in an underestimation of users, potentially obscuring significant effects of Cbn use on fetal growth. According to authors, 42% of women w/ at least 1 positive urine test reported Cnb use 3 or more times per wk, compared to 19% of women w/ negative urine tests. Strengths: Performed repeated interviews and urine testing, as well as separate analyses for self-report and urine testing. Interviewers were unaware of the results of infant assessments at the

Study/ Location	Study Design, Sample Sizes, Statistical Analysis	Outcomes of Interest	Exposure Measurement Methods	Exposure quantification	Results	Covariates/ Confounders	Comments
							time of the postpartum interview. The study controlled for the use of cocaine in the analyses. <u>Limitations:</u> Analyses were not performed using the 2 measures of Cnb use combined (i.e., self-report and urine testing) The sample size for analyses of congenital anomalies was very small.

Human Studies Birth Outcomes

Studies that were not as informative as those tabulated in the table above are summarized below in paragraphs

Astley et al. (1992)

Astley et al. (1992) is a retrospective cohort study of health maintenance organization members who received prenatal care between 1982 and 1984. The sample comprised 40 exposed women (prenatal cannabis users) and 40 unexposed women who were group matched to the exposed women based on alcohol consumption before and during pregnancy and infant's sex, race, and date of birth. Cannabis exposure was assessed through interviews conducted 1 month post-partum and was quantified as joints per day. Frequency of cannabis use was classified as no use, 1 to 3 times/wk (n=15), 3 to 4 times/wk (n=11), every day (n=14), 2 to 5 times/d (n = 15), and 10 times/d (n=1), for each trimester; however, analyses reported results for cannabis as exposed vs. unexposed. A dysmorphologist assessed children for facial features resembling fetal alcohol syndrome (FAS) between the ages of 5 and 7 years. The dysmorphologist classified children into five groups, varying from no unusual features, to definite FAS. Exposed children were not more likely to have FAS-like facial features or non-FAS associated minor anomaly triangles or patterns. No child was classified as having a definite FAS-like face.

Berenson et al. (1996)

Berenson et al. (1996) conducted a retrospective cohort study to examine effects of prenatal care on infants born in 1989 and 1990 to women who use drugs. Medical records for all deliveries in the study period were reviewed for drug screen results, neonatal complications, and maternal characteristics. Alcohol and cigarette use were assessed in structured interviews at intake, and urine drug screening was performed for women who admitted drug use in the past or having a partner who used drugs. All women who presented to the labor and delivery unit without having had prenatal care were also screened by urinalysis. Women who tested negative for all drugs (n=147) and women who tested positive for Cnb (n=36) and/or other known drugs (n=55) were included. Maternal Cnb use, adjusted for race, cigarette, and alcohol use, was not significantly associated with birth outcomes: LBW OR=1.2 (0.5, 2.8), small head size OR=0.8 (0.3, 1.8), infant ICU admission OR=0.5 [reported as 5] (0.2, 1.4), fetal distress OR=1.6 (0.6, 4.2), among others. Cocaine and/or multiple drug use were also associated with complications, and Cnb use was associated with other maternal characteristics that might not have been considered in analyses, so the associations with Cnb may be confounded by other factors.

Ewing et al. (1997)

Ewing et al. (1997) conducted a case-control study to examine the association between paternal age and isolated membranous VSD. There were 491 cases, defined as infants born alive during 1981-1989 to a resident of the study area and diagnosed with isolated membranous VSD by the age of one year, and 3549 randomly selected control infants born in the same years and study area and having no diagnosis of congenital heart disease. Maternal cannabis use during the three months before conception through the first trimester and paternal cannabis use during the six months before conception were analyzed as binary variables. Maternal cannabis use, and alcohol and cigarette use by either parent were not significantly associated with VSD, and were not included in the regression model. Paternal cannabis use was associated with VSD: **OR=1.36 (1.05, 1.76)**, adjusted for maternal cocaine use, maternal age, infant sex and race, and interaction between paternal age and paternal cocaine use.

Klonoff-Cohen and Lam-Kruglick 2001

Klonoff-Cohen and Lam-Kruglick (2001) conducted a case-control study to investigate whether maternal and paternal drug use during conception, pregnancy, and postnatally increase the risk of sudden infant death syndrome (SIDS; death between 1 wk and 1 yr of age). The sample comprised 239 autopsy-confirmed SIDS cases identified from health department records, and 239 control infants who were born in 1989-1992 in the same 110 hospitals as cases and matched to cases by birth date, sex, and race. Parents were interviewed by telephone to ascertain timing and frequency of use of cigarettes and alcohol, and recreational and other drugs during 3 periods: conception (defined as the time between last menstrual period and confirmation of pregnancy), pregnancy, and postnatal. Associations between SIDS and maternal and paternal cannabis use are shown in the table. The authors found no interactions among paternal drug use, smoking, and drinking, and maternal and paternal recreational drug use.

Maternal and Paternal Cnb use and SIDS: OR (95% CI), number of users, by exposure period

Exposure period	Maternal Cnb use	Paternal Cnb use
Conception	1.1 (0.6, 2.0)* n=53	**2.2 (1.2, 4.2)**† n=75
Pregnancy	0.6 (0.3, 1.6)* n=28	2.0 (1.0, 4.1)† n=58
Postnatal	0.6 (0.2, 1.8)* n=21	**2.8 (1.1, 7.3)**‡ n=30

* Adjusted for maternal tobacco smoking during pregnancy

† Adjusted for paternal postnatal tobacco smoking and paternal alcohol use during conception

‡ Adjusted for paternal postnatal tobacco smoking

Lozano et al. (2007)

Lozano et al. (2007) is a prospective cohort study that examined the relationship between prenatal cannabis exposure and newborn weight, length, and HC using samples from the Meconium Project cohort in Barcelona, Spain. The authors obtained 974 meconium samples that had been collected at 24 hours after delivery and analyzed them for Δ^9-THC and metabolites using a GC-MS-based assay. Of these, 52 (5.3%) were positive for cannabis, with eight also being positive for cocaine and/or opiates. Forty-six samples tested positive for other drugs and were excluded from further analyses, leaving n=928. After adjustment for tobacco smoking, infant sex, maternal age, and gestational age, there were no statistically significant associations between prenatal cannabis exposure and newborn weight, length, or HC.

Molar et al. (2018)

Molnar et al. (2018) conducted this prospective cohort study to examine secretory immunoglobulin A (SIgA) in 5-year old children whose prenatal exposure to cannabis and cigarettes had been assessed. Low levels of SIgA may indicate a poorly functioning immune system, and high SIgA levels may indicate chronic infections and continuing exposure to environmental toxins. SIgA was assayed using samples of passive drool collected from 45 children for whom prenatal cannabis and cigarette exposure had been assessed using a combination of interviews, maternal saliva, and infant meconium samples. The three prenatal exposure categories were cigarette and cannabis use (n=17), cigarette use only (n=16), and no use (n=12). Cannabis and cigarette use was associated with higher SIgA levels at age 5 (p=0.006), but no dose-response relationship was determined. The researchers did not analyze cannabis by comparing the cigarette only group to the cigarette and cannabis group (prenatally or postnatally), but they indicated that *postnatal* cigarette exposure (assessed by child's saliva cotinine levels) was associated with the highest levels of SIgA when postnatal maternal cannabis use was relatively high (*b* =12.38 [CI 3.66, 21.09], p=0.007), an association not observed when postnatal maternal cannabis use was low. The effects of prenatal and postnatal cigarette and cannabis exposure are difficult to distinguish because most women who used cigarettes and cannabis prenatally continued postnatally. There was no significant interaction between prenatal cigarette and cannabis use (p=0.10). The authors concluded that "prenatal cigarette and the combination of prenatal cigarette and cannabis exposure were both associated with higher SIgA levels (vs. control group)" in these children.

Scragg et al. (2001)

Scragg et al. (2001) conducted this case-control study in New Zealand to determine whether maternal cannabis use is associated with SIDS (defined as death between day 28 and 1 year) in 1987-1990. Of 485 SIDS cases, the 393 with obstetric records were interviewed. Of 1800 controls randomly chosen from hospitals to represent the study region, 1592 were interviewed. Mothers were interviewed in their homes and asked if

they smoked cannabis during pregnancy and since the birth of the baby, and the frequency of use. They were also asked about use of other substances. There were 174 women who used cannabis during pregnancy and 1803 who did not. After adjustment for ethnicity (Maori, Pacific Islander, other) and tobacco use, cannabis use during pregnancy was associated with SIDS: **OR=1.60 (1.13, 2.27)**. However, the OR diminished and was ns when the model also included the other "main confounders", SES, marital status, age at 1st pregnancy, and infant age: OR=1.30 (0.69, 1.87). The association was smaller when additionally adjusted for region, time of day, season, age mother left school, maternal age, parity, attendance at antenatal clinic and education classes, infant sex, BW, gestation, sleep position, breastfeeding, and bed sharing: OR=1.18 (0.76, 1.85). The OR for prenatal cannabis use may be confounded by maternal cannabis use since delivery, which was a slightly stronger predictor of SIDS, particularly for those who used weekly or more frequently.

Varner et al. (2014)

Varner et. al. (2014) conducted this case-control study of stillbirths (SB) in multiple sites as part of the Stillbirth Collaborative Research Network. The authors attempted to enroll all eligible women who delivered SBs at ≥18 wks, and a representative sample of women who delivered live births at ≥20 wks gestation, with oversampling of live births at <32 wks and women of African descent delivering ≥32 wks. The sample comprised 418 stillborn fetuses and 1050 eligible live births with cord blood/maternal serum screening. A perinatal pathologist performed a comprehensive standardized fetal postmortem examination on cases. Umbilical cord, maternal blood, and other biological samples were collected. Umbilical cord homogenate was analyzed for tetrahydrocannabinolic acid (THCA) and other drugs/metabolites, and confirmed by mass spectrometry, and maternal serum was analyzed for cotinine. Women were interviewed about illicit drug use, though the authors appear to have relied solely on cord blood screening to identify Cnb exposure. The authors weighted statistical analyses for oversampling and other aspects of the study, and adjusted for confounding using a risk factor score calculated from demographic, socioeconomic, medical, and other risk factors, excluding smoking status and illicit drug use. The adjusted OR for stillbirth and THCA was **2.34 (1.13, 4.81), p=0.021**. When the analysis was limited to non-anomalous, singleton pregnancies, the OR was **2.83 (1.34, 5.99), p=0.007.** Adjusting for cotinine levels reduced the OR for THCA (OR not reported), but adjusting for THCA did not reduce the OR for cotinine. The OR for cotinine and stillbirth was 2.25 (1.59, 3.19). Thus, authors could not exclude the possibility that association between Cnb and stillbirth is partially due to confounding by tobacco smoke (OR not reported).

Appendix 2. Human Developmental Studies: Neurodevelopmental Outcomes

Appendix Table 2.1 Human Neurodevelopmental Studies: CNS Maturation

Study/ Location	*Study Design, Sample Sizes, Statistical Analysis*	*Outcomes of Interest*	*Exposure Measurement Methods*	*Exposure quantification*	*Results*	*Covariates/ Confounders*	*Comments*
Dahl et al 1995 Pittsburgh, PA MHPCD	Prospective cohort Participants selected from 3 cohorts N = 1360 for 1st and 2nd cohorts Women were selected for the 3 cohorts using the following criteria regarding substance use during preg: 1st: drank ≥3 alcoholic drinks/wk 2nd: used ≥2 joints/mo 3rd: drank ≥1 drinks/ day or used ≥1 joint/ day during 1st trimester For each cohort, the next woman who reported less alcohol or Cnb use was also selected 1st and 2nd cohort combined: n = 763, 3rd cohort: n = 108 n = 38 for current study 19 subjects from first two cohorts and 19	Sleep variables from polysomnographic recordings at 3 yo. Studies were conducted in a comfortable, child-oriented sleep laboratory All children were healthy and free of medications Children slept on their usual (home) schedule w/ a parent present in the sleep laboratory 3 nights. Data were collected by staff who were blind to exposure status. 1st night was considered an adaptation night. Analyses use 2nd and 3rd night data	Maternal self-report at 4th and 7th mo of preg and at delivery for each mo of 1st trimester and each trimester Bogus pipeline technique used	Exposed: 1st trimester Cnb use ≥1 joint/ wk, group avg 2.8 joints/day, n=18 Unexposed: 1st trimester Cnb use <1 joint/ mo, group avg<0.01 joints/day, n=20 Hashish considered equivalent to 3 joints and sinsemilla to 2 joints	1st trimester Cnb exposure was assoc w/ lower sleep efficiency (% of recording period spent asleep) (mean ± SD): Exposed: **91 ± 3.8** Unexposed: **94.4 ± 2.1, p<0.05** **Spearman's ρ=-0.41, p<0.01** Cnb exposure was assoc w/ more frequent arousals after sleep onset: Exposed: **8.2 ± 5.3** Unexposed: **3.2 ± 4.6, p<0.005** **Spearman's ρ=0.46, p<0.004** Cnb exposure was assoc w/ more awake time after sleep onset: Exposed: **27.4 ± 20.0** Unexposed: **13.7 ± 12.4, p<0.05** Differences in number of minutes in each sleep stage, latency to rapid eye movement period, total sleep time, bedtime, or wake-up time for exposed vs. unexposed groups were ns	There were no ss differences btwn groups in alcohol, nicotine, or other substance exposure, or any major demographic variables (only difference: exposed mothers had fewer stressful life events). Analyses using these variables as covariates yielded the same pattern of results as analyses that dropped subjects with other substance exposures. <u>Considered</u>: Postnatal Cnb use Other drug use Education	Authors state the specificity of the observed sleep changes is consistent w/ a physiologic cause. Further, the assoc w/ only 1st trimester Cnb is consistent w/ a teratogenic model. Current findings' also consistent w/ previously reported sleep disruptions at birth and increased body movements during newborn sleep Data from neonatal sleep studies were available for 31 children in this study. Comparing sleep disruptions in 3 yo w/ data from neonatal recordings, neonatal small body movements were correlated w/ number of arousals at 3 yrs, Spearman's ρ=0.67, p>0.006. <u>Strengths</u>: Prospective design Bogus pipeline

subjects from 3rd cohort were selected Children whose mothers used either ≥1 joint/wk or <1 joint/mo during 1st trimester were selected; no other selection criteria or methods reported Mothers and offspring were assessed at 8, 18, and 36 months for substance use; demographic, lifestyle, and psychosocial info Mann-Whitney *U* test, Spearman's rank correlations, Fisher's Exact test, Student's *t* test			Correlations btwn sleep efficiency and number of arousals and 2nd and 3rd trimester Cnb use were ns	Social support Mother's perception and expectations of child Mother's psychiatric status	<u>Limitations</u>: Small sample size

Study/ Location	*Study Design, Sample Sizes, Statistical Analysis*	*Outcomes of Interest*	*Exposure Measurement Methods*	*Exposure quantification*	*Results*	*Covariates/ Confounders*	*Comments*
Fried and Makin 1987 Ottawa, Canada OPPS	Most births 1980 - 1983 Original sample n = ~700 women Eligibility criteria: Used Cnb during preg Heavier social drinkers Regular cigarette smokers, Additional 50 women who did not use Cnb or cigarettes, and who drank little or no alcohol were selected for follow-up One infant was randomly selected from each set of twins (5) n = 250 term infants (135 males, 115 females) Canonical correlations, multiple regression	Neonatal behavior (NBAS) NBAS assessment on days 3-6 by 2 trained testers who were unaware of mothers' drug use	Maternal-self report Repeated interviews asking about drug use and 24-h diet recall in the previous trimester of preg 50 Cnb/alcohol/ smoking abstainers 203 Cnb non-users 23 women smoked <1 joint/wk over preg 24 women smoked ≥1 joint/wk	Avg Cnb use over preg, joints/wk No. of hashish joints was multiplied by 5 Cigarette use, a nicotine score was determined by multiplying no. of cigarettes smoked by the nicotine content of the specific brand	Multiple regression of Cnb use and NBAS measurements: Partial F test results: <u>Startles</u> (increased) **p=0.000** <u>Response to light</u> (reduced) p=0.220 <u>Habituation to light</u> (reduced) **p=0.032** <u>Tremors</u> (increased) p=0.05 <u>Irritability</u> (increased) p=0.053 No ss results for Cnb w/ habituation to sound, response to sound, miscellaneous sounds, lability of state**,** consolability, smiles, self-quieting Diminished responsiveness to light was not uniquely assoc w/ prenatal Cnb exposure after drug use was considered, but caffeine, nicotine and Cnb considered together were ss predictors of this variable (overall R=.21, **p =0.015**)	<u>Included</u> Alcohol Nicotine Caffeine (mg/day) GA (wks) Age of infant at testing (days) Family income Maternal age <u>Considered</u> Type of delivery Sex of infant Obstetric medication Gravidity	Predominantly middle class, low-risk population. <u>Strength</u> Cnb was analyzed as a continuous variable. Used canonical analysis to determine which Brazelton variables were assoc w/ prenatal Cnb use. Authors noted that this reduced the risk of Type I errors and accommodated the intercorrelation of drug use measures.

Study/ Location	*Study Design, Sample Sizes, Statistical Analysis*	*Outcomes of Interest*	*Exposure Measurement Methods*	*Exposure quantification*	*Results*	*Covariates/ Confounders*	*Comments*
Scher et al. 1988 Pittsburgh, PA MHPCD	Prospective cohort Participants were recruited from prenatal clinic at Magee-Womens Hospital 1982 – 1985 N = 763 live singleton births to women who were selected on basis of Cnb or alcohol use <u>Selection for current study:</u> Mother consumed ≥1 drinks or Cnb joints/day during 1st trimester, and next birth to mother who used less alcohol or Cnb n = 55 2- to 2½-h EEG-sleep recording was obtained 24 to 36 h after birth on swaddled infant 45 min to 1 h after morning feeding. Recordings were scored independently by electroencephalo-grapher who was unaware of prenatal substance exposure Multiple regression, ANCOVA	Disturbances in neonatal sleep cycling, motility, and arousal measured w/ EEG-sleep recording obtained 24 to 36 h after birth Recordings were scored for EEG state, rapid eye movements, arousals and body movements in 1-min scoring epochs Scoring was based on operational definitions using representative neonatal EEG recording samples Sleep behaviors including indeterminate or transitional sleep, arousals, and phasic rapid eye movement activity also included in scoring manual	Maternal self-report Interviewed during 4th mo of preg to assess alcohol, Cnb, tobacco, and other drug use for the yr before preg and 1st trimester 2nd interview at 7th mo and 3rd interview at delivery assessed substance use during 2nd and 3rd trimesters, respectively	Cnb exposure expressed as avg joints/day for regression analyses To compare adjusted means of sleep variables, 1st trimester Cnb use was dichotomized: *Users* used Cnb at least once/day (n=11) Abstainers (n=29)	Prenatal Cnb in each trimester assoc w/ increased body movements, decreased total quiet sleep, and decreased trace alternant quiet sleep. Cnb in different trimesters assoc w/ increased mixed active sleep, decreased low voltage irregular active sleep, and fewer rapid eye movements EEG outcomes: Regression analysis results by trimester exposed (R^2, standardized β) and ANCOVA adjusted means for 1st trimester Cnb users and abstainers *Small body movements* 1st: 0.10, 0.31 3rd: 0.13, 0.36 Abstainers: **0.02** Users: **0.2, p<0.05** *Large body movements* 1st: 0.25, 0.50 2nd: 0.12, 0.34 3rd: 0.33, 0.57 Abstainers: **0.2** Users: **0.6, p<0.01** *Total quiet sleep* 1st: 0.23, -0.41 2nd: 0.18, -0.43 3rd: 0.15, -0.36 Abstainers: **27.8** Users: **15.1, p<0.05** *Trace alternant quiet sleep*	<u>Included</u>: Alcohol use Tobacco use Maternal age Maternal education Income Race Marital status Other illicit drug use Infant sex BW Dubowitz score Ponderal index EEG technician Interactions w/ tobacco, alcohol were ns	Authors note that noradrenergic neurons are present and presumably functional throughout fetal life, and speculate prenatal Cnb exposure may contribute to overelaboration of noradrenergic system that is reflected in increased motility and longer sleep segments Sample was 47% African American and 53% White <u>Limitations</u>: Sample came from a low SES, urban population so results may not be generalizable to other groups

Study/ Location	Study Design, Sample Sizes, Statistical Analysis	Outcomes of Interest	Exposure Measurement Methods	Exposure quantification	Results	Covariates/ Confounders	Comments
					1st: 0.22, -0.46 2nd: 0.16, -0.32 3rd: 0.12, -0.34 Abstainers: **23.8** Users: **11.6, p<0.01** *Mixed active sleep* 1st: 0.08, 0.29 3rd: 0.09, 0.30 Abstainers: 26.4 Users: 31.3, p≥0.05 *Low voltage irregular active sleep* 1st: 0.10, -0.33 Abstainers: **19.7** Users: **6.7, p<0.05** *REM (number/min)* 1st: 0.09, -0.32 Abstainers: **4.6** Users: **2.5, p<0.05** *Indeterminate sleep* Abstainers: **24.8** User: **40.1, p<0.01**		

Study/ Location	***Study Design, Sample Sizes, Statistical Analysis***	***Outcomes of Interest***	***Exposure Measurement Methods***	***Exposure quantification***	***Results***	***Covariates/ Confounders***	***Comments***
Scher et al. 1998 Pittsburgh, PA MHPCD	Prospective cohort 1986-1987 Subjects recruited from a large university-affiliated obstetric hospital All women who drank ≥1 alcoholic drink/day in the 1st trimester and all women who used ≥1 joint/day, and the next woman interviewed who drank less or used less Cnb were enrolled n = 74 Term infants w/ no medical complications were selected for visual evoked potential (VEP) studies (no further details were provided on sample selection) Flash VEPs were obtained at 24-36 hrs after birth and 1 mo, and pattern VEPs at 4, 8, and 18 mos. All VEPs were scored by the same neurophysiologist who was blind to exposure Multiple regression	VEPs w/ monocular and binocular flash and pattern testing to determine possible maturational changes in components of VEP in the absence of neonatal behavioral disturbances Only binocular data were analyzed and reported VEP binocular flash testing: 24-36 hours after delivery (n=22) 1 mo (n=18) VEP pattern testing: 4 mos (n=33) 8 mos (n=58) 18 mos (n=70) N_1 = 1st major negative wave on VEP P_1 = 1st major positive wave N_2 = 2nd major negative wave Wave latency (N_1, N_2, P_1) and amplitude (N_1–P_1) were measured	Women interviewed during 4th or 5th mo of preg to ascertain use of Cnb, tobacco, alcohol, and other illicit drugs for substance use in the yr prior to preg and 1st trimester use; 7 mos for 2nd trimester use; 24 hours after delivery for 3rd trimester use Women interviewed and infants physically examined at 8 and 18 mos	Cnb use levels (% in 1st, 2nd, and 3rd trimester): None (58.1, 69.9, 68.9) Light, >0-3 joints/wk (16.2, 19.2, 18.9) Moderate, 4-6 joints/wk (4.1, 4.1, 5.4) Heavy, ≥1 joint/day (21.6, 6.8, 6.8)	Effect of Cnb was mostly related to 3rd trimester exposure Regressions for binocular VEP testing and trimester of Cnb exposure: 1st trimester: Decreased N_1–P_1 amplitudes at 18 mos (R^2 = -0.15) 2nd trimester: Increased N_1 latency at 1 mo (R^2 = +0.39) 3rd trimester: Increased N_1 (R^2 = +0.44) and P_1 latencies at 1 mo (R^2 = +0.56) Decreased P_1 latency at 8 mos (R^2 = -0.12) Increased P_1 latency at 18 mos (R^2 = +0.22) Prolonged latencies of P_1 waveform suggest a delay in brain maturation of visual function Reasons for N_1–P_1 amplitude changes are not apparent	<u>Included</u>: Maternal age Education Income Race Marital status Infant sex BW Dubowitz score Alcohol Tobacco Other illicit drugs	Subjects in this report appear to be distinct from subjects in other MHCPD reports Authors state high frequency wavelets appear only after 1 mo, which can help explain how substance use can affect latencies observed at 1 mo and later ages but not birth Authors state the data suggest that prenatal substance exposure causes a transient delay in the neurophysiologic maturation of the visual system, particularly during infancy, for the P_1 wave. Delays in the maturation of other components of the VEP suggest that delays in brain maturation persist, depending on the substance, time of exposure, and age at test <u>Limitation</u> Lack of repeated longitudinal testing for some patients

Study/ Location	*Study Design, Sample Sizes, Statistical Analysis*	*Outcomes of Interest*	*Exposure Measurement Methods*	*Exposure quantification*	*Results*	*Covariates/ Confounders*	*Comments*
Tansley et al. 1986 Ottawa, Canada OPPS	N=~700 Pregnant women, recruited through obstetricians' offices and the media, provided information through multiple interviews. Offspring were assessed multiple times for various outcomes Children of mothers who had used Cnb, or were passively exposed to Cnb, or who had smoked ≥1 pack of cigarettes/day during preg were selected. For Cnb controls, non-exposed children were matched to exposed by age and approximate prenatal nicotine and alcohol exposure. Nicotine controls were matched by age and maternal alcohol use. n = 101 children Observations were conducted btwn 36 and 120 mos of age [see comment]; avg was 48.75 mos	Transient pattern-evoked visual cortical potential (TPVCP) as a measure of maturation of visual mechanisms Visually-evoked responses were examined for latency and amplitude of major components To measure binocular interaction to compare developmental status of visual pathways, authors developed a "binocular index" for both latency and amplitude: The binocular index for latency increases when the latency of the binocular pattern response decreases relative to the monocular response The binocular index for amplitude decreases with increases in the amplitude of the binocular pattern response	Mothers self-reported prenatal Cnb use in detailed interviews, repeated during preg Cnb urine screen to complement self-report (mentioned in discussion but not described)	No Cnb use during preg (69.0%) Any Cnb use during preg (31%)	96 children completed some of the visually-evoked response trials Mean latencies for each of the components measured for the control groups for left eye stimulation, right eye stimulation, and binocular stimulation showed good correspondence with published norms. For both monocular and binocular conditions, a slightly greater proportion of exposed children had longer P1 latencies than expected based on avg for the control groups (no statistics reported) Although avg binocular index values for exposed and control groups were close, there was more variability of binocular indices for both the latency and amplitude among Cnb- and nicotine-exposed group ($F=2.08$, $df = 25,25$, $p<0.05$) Low correlation btwn latency and amplitude across groups suggest they are independent ($r = 0.045$.) Tighter clustering of data with higher levels of both binocular latency and amplitude indices in exposed group but not controls (no statistics reported)	Cnb-exposed and unexposed children were matched by age and prenatal nicotine and alcohol exposure	Authors state finding of greater variability among Cnb and nicotine exposed children is consistent with reduction in rate of central nervous system maturation. Authors mention probable uncontrolled confounding by postnatal environmental smoke exposure Authors note the potential for outcomes to manifest as short or long-term, or not at birth, but still influence development and result in neurophysiological changes in the future Subjects were reportedly up to 120 mos of age; however recruitment for OPPS began in 1978, which is 8 yrs before the publication of this report

Appendix Table 2.2 Human Neurodevelopmental Studies: Attention

Study/ Location	*Study Design, Sample Sizes, Statistical Analysis*	*Outcomes of Interest*	*Exposure Measurement Methods*	*Exposure quantification*	*Results*	*Covariates/ Confounders*	*Comments*
El Marroun et al. 2011 Rotterdam the Nether-lands Gen-eration R	Prospective cohort N = 5,512 <u>Eligibility Criteria:</u> Resident in the study area "Are at their delivery date" Delivery date btwn April 2002-January 2006 Information on child behavioral problems at 18 mos available n = 4,077 Mothers in non-participating group were younger, less educated, less often of Dutch national origin, and had higher psychopathology symptom scores than the mothers in the participating group Linear regression and logistic regression models	Behavior and emotional problems at 18 mos Measured using the Child Behavior Checklist for toddlers (CBCL) filled out by mother at 18 mos of age and focused on 3 specific subscales: Anxious and/or depressed Attention problems Aggressive behavior	Maternal self-report Women were mailed 4 questionnaires during preg and their partners were mailed 1 questionnaire	Mother reported information on timing of Cnb use (never, use before preg, stopped after knowledge of preg, continued use through preg) and frequency of use (daily, weekly, monthly) Exposure was categorized as: Cnb exposure in preg, mostly w/ co-use of tobacco during preg Tobacco-only exposure in early preg Tobacco-only exposure throughout preg Non-use of Cnb or tobacco during preg	Linear regression models assessing assoc btwn maternal Cnb use and child scores: β (95% CI) Aggressive behavior: Total 0.91 (-0.22–2.04), p=0.11 Boys: -0.15 (-1.65–1.35) p=0.84 Girls: **2.02 (0.30–3.73) p=0.02** Attention problems: Total: 0.36 (-0.02–0.74) p=0.06 Boys; -0.20 (-0.69–0.30) p=0.43 Girls: **1.04 (0.46–1.62) p<0.001** Anxious and/or depressive problem: Girls: -0.02 (-0.40–0.45) p=0.91 Boys: -0.36 (-0.73–0.01) p=0.06 Logistic regression analyses w/ a cutoff score of the CBCL in girls: OR (95%CI) Aggressive behavior: 1.66 (0.38–7.26) p=0.50	<u>Included:</u> Age and gender of child Parental education, national origin, psycho-pathology Paternal models also corrected for maternal Cnb and/or tobacco use <u>Considered:</u> Alcohol use Other drug use	Gestational exposure to Cnb was assoc w/ aggression and attention problems at 18 mo in girls only. Long-term tobacco exposure was assoc w/ similar behavioral problems. No effect in boys. Maternal Cnb use during preg could also have concurrent tobacco use. The logistic regression w/ a cutoff score was only conducted in groups w/ ss effects demonstrated in the linear regression models, therefore there are no data for the boys or total groups from logistic regression. Paternal use did not predict aggression and attention in offspring. Authors postulate that this is due to a biological mechanism in the mother. <u>Strengths:</u> Agreement btwn maternal self-report and urinalyses

Cnb exposure in preg (n=88) mostly w/ co-use of tobacco during preg	Attention problems: **2.75 (1.27–5.96) p=0.01** Linear regression models assessing assoc btwn paternal prenatal Cnb exposure and child scores: β (95% CI) Aggressive behavior scores: Total 0.54 (-0.06–1.14) p=0.08 Boys: 0.54 (-0.32–1.39) p=0.22 Girls: 0.59 (-0.26–1.44) p=0.17 Attention problems Total:0.09 (-0.11–0.29) p=0.40 Boys:0.14 (-0.14–0.43) p=0.31 Girls: 0.02 (-0.27–0.30) p=0.92	was good (Yule's *Y*= 0.77). Self-reported Cnb use was in agreement w/ national figures among Dutch women aged 15-64 yrs (recent use 1.5%, current use 3.1%) in the same period. <u>Limitations:</u> Use of mother's reports of child's behavior. Authors note that response analyses showed mothers who were non-responders were at a higher risk for Cnb use during preg. According to the authors this indicates the study may have underestimated the links btwn maternal Cnb use and negative offspring outcomes. Although the study collected information on timing of use and amount of Cnb use, this information was not included in the analysis presented in the publication.

Study/ Location	*Study Design, Sample Sizes, Statistical Analysis*	*Outcomes of Interest*	*Exposure Measurement Methods*	*Exposure quantification*	*Results*	*Covariates/ Confounders*	*Comments*
Fried and Watkinson 2001 Ottawa, CA OPPS	Prospective cohort Most births in 1980 - 1983 N = 698 pregnant women volunteers recruited through obstetricians' offices and the media <u>Eligibility criteria for follow-up cohort:</u> Children whose mothers reported any use of Cnb during preg, drank avg >0.85 oz absolute alcohol /day, smoked avg ≥16 mg nicotine/day during preg (n = 140) Children whose mothers were nonusers of Cnb, abstained, or drank little alcohol, or were nonsmokers (n = 50) Exclusions: family moved out of area, withdrew from study, unavailable for testing, medicated for attention deficit disorder n = 152 Principal components analysis, ANOVA	Facets of attention in adolescents (13-16 yo), using 11 tests CPT Conners version Wisconsin Card Sorting Test (WCST) Stroop Test interference score Wechsler Intelligence Scale for Children (WISC) - arithmetic subtest Sentence Memory Test Seashore Rhythm test Knox cube Test WISC-III subtests Picture Arrangement, Arithmetic, Block Design; Vocabulary subtests were transformed into a Wechsler Short Form Deviation Quotient to estimate full-scale IQ	Maternal self-report Interviews conducted in woman's home during each trimester after enrolling in study and after delivery Interviews of mothers collected data on quantity and pattern of drug and alcohol use, caffeine use, age, height, pre-preg weight, weight gained during preg, maternal passive cigarette smoke exposure, general health, previous pregs, 24-hour dietary recall, father's medical history and level of education, and SES	Avg number of Cnb joints/wk across preg categorized into 3 groups: No use Infrequent/ moderate use: >0 and <6 joints/wk Heavy use: ≥ 6 joints/wk	5 factors of attention, based on the tests: Shift/flexibility: ability to move attention across stimuli over time (WCST) Encode/retain: a measure of working memory (Knox Cube, Sentence Memory, Seashore, WISC-Arithmetic) Impulsivity: ability to maintain attention over time (CPT reaction time and commissions) Stability: consistency of attentional effort over time (CPT reaction time and omissions) Focus/execute: capacity to allocate attentional resources to a specific task while screening out irrelevant stimuli (Stroop) Heavy prenatal Cnb use was ss assoc w/ poorer performance on stability of attention over time: factor scores of heavy Cnb users' offspring reflected more omissions and less consistent reaction time over blocks of the task (**F(2,147)=5.2, p<0.01**) Assoc btwn prenatal Cnb and other factors were ns in unadjusted models; adjusted results were not reported	Variables examined for assoc w/ drug of interest at p≤0.10. Variables assoc w/ relevant component scores at p≤0.05 were retained as covariates <u>Included:</u> Prenatal alcohol or tobacco exposure <u>Considered:</u> Maternal age at birth Avg level of parental education Family income (current and during preg) Current maternal drug use Prenatal passive smoke exposure Passive smoke exposure of adolescent Sex of adolescent Parents together or separated Adolescent's smoking habits (w/ urine cotinine confirmation)	Current sample was similar to larger cohort w/ respect to mother's age at delivery, income, parity <u>Strengths:</u> Authors note that multiple interviews throughout woman's preg allow for evaluation of consistency of self-report <u>Limitations:</u> Authors state the focus/execute factor may be a poorly defined construct with only the Stroop test as a measure Authors also state that no direct, concurrent measures of attentional deficits in either parent were available in present work

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Fried et al. 1992a Ottawa, Canada OPPS	Prospective cohort N = 698 <u>Eligibility Criteria:</u> Children of women who reported any use of Cnb during preg Children of women who drank alcohol beyond a daily average of 0.85 oz Children of women who smoked an average of ≥16 mg nicotine/day during preg Children of 50 women who were nonusers of Cnb, who abstained or drank little alcohol, and who were nonsmokers n = 190 Some participants were unavailable due to: families moving out of Ottawa area, withdrew from study, or children unavailable for	Attentional behavior including impulsivity in 6 yo Assessed by: <u>Attentional dependent memory scores</u> Sentence Memory Test Memory subscale of the McCarthy Scales of Children's Abilities Target Test <u>Impulse control and sustained attention</u> Gordon Diagnostic System using 3 delay task scores and 3 vigilance task scores <u>Observational measures relevant to attentional behavior</u> Impulsive-Hyperactive scale of Conners' Parent Rating Scale-48 (CPRS-49) completed by mother at time of testing)	Maternal self-report by interview	Maternal average Cnb use across preg categorized into 3 groups: No or infrequent use (<1 joint/wk) (n=93) Moderate use (>1 and <6 joints/wk) (n=14) Heavy use: (≥ 6 joints/wk) (n=19)	Stepwise DFA resulted in one significant function (**chi square = 16.7, p<0.05, Wilks's lambda =0.87, 73.0% correct classification**) and accounted for 88% of the discriminatory variance. This assoc was observed to be dose-related, w/ maximal separation btwn the heavy user group and the no/ infrequent use group The primary variables related to this discriminant function were (structure coefficient, disciminant function coefficient): - Total correct on Gordon vigilance task (0.60, 0.96) -No. of omissions on Gordon vigilance task (-0.60) Impulsive/Hyperactive scale of CPRS-48 (-0.46, -0.42) Cross-validation analysis compared classification of 2/3 sample w/ the other 1/3 and indicated that the drug/outcome assoc found via DFA were stable After controlling for mother's age at delivery and average level of parental education the negative assoc btwn prenatal	<u>Included (in MANCOVA):</u> Mother's age at delivery Average level of parental education <u>Considered:</u> Family income Maternal pre-preg weight Mother's caffeine intake and nutrition during preg Preg difficulties Parity Offspring sex Predominant language spoken by offspring Parental relationship Alcohol and cigarette use Home environment (assessed using Home Inventory)	Authors note that the increase in omission errors and greater number toward the end of the vigilance task reflect a deficit in sustained attention. However, interpretation of inattention problems in children cannot be accurately made because of the lack of assoc btwn memory tasks and prenatal Cnb use in this study. The specific outcomes assoc with deficits from prenatal tobacco exposure differed from those deficits assoc with prenatal Cnb exposure. <u>Strengths:</u> Assessed potentiating effects of multiple drugs using factorial analyses of covariance (ANCOVAs). Authors note the use of the same interviewer throughout allowed a reliability check and enhanced rapport btwn subject and interviewer. <u>Limitations:</u> Authors note that fetal exposure to Cnb was measured in terms of

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	testing; one child excluded because he was receiving medication to control a behavior problem n = 126 No differential loss of subjects w/ respect to drug variables Multivariate analysis, ANCOVA, MANOVA, Stepwise Discriminant Function Analysis				Cnb use and outcomes remained significant: **F(8, 238) = 2.1, p<0.05, one-tailed** Examination of delay and vigilance scores revealed ns variation across Cnb use groups Examination of total correct and omissions scores of the vigilance task show that a ns btwn groups effect and a ns interaction “appear to contribute substantially to the discriminant function” demonstrating a dose-response assoc w/ Cnb usage w/in each time block The no. of omission errors increased btwn 1st and last block by 13% in children in heavy Cnb use group compared to 3% in the moderate group and 4% in the no/infrequent use groups		average maternal use over preg and does not distinguish timing of exposure or account for sporadic heavy use during preg. Authors state that some factors that may affect child’s attention such as maternal personality or parenting style were not tested.

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Fried et al. 1998 Ottawa, Canada OPPS	Prospective cohort Most births 1980 - 1983 N = 698 Pregnant women were recruited through obstetricians' offices and public media and interviewed Follow-up cohort initially included 140 children of women who: used Cnb, consumed >0.85 absolute alcohol/ day, or smoked ≥16 mg nicotine/day during preg, and 50 children of women who used no Cnb or nicotine, and little or no alcohol n=131 at 9-12 yo Exclusions and losses: family moved away, unavailable for testing, withdrew from study, French language, child taking Ritalin Age (n): 9 (3), 10 (70), 11 (34), 12 (24) Children were administered a large battery of tests, from which measures of cognitive and executive functioning were selected for the current analyses. Assessments were	Cognitive and executive function in 9-12 yo children, measured by: Wechsler Intelligence Scale for Children-III (WISC-III) w/ its subscales, composite, and derived scores Gordon Diagnostic Delay and Vigilance Tasks Category Test Auditory Working Memory Test Fluency Test Tactual Performance Test (total time to perform) Developmental Test of Visual-Motor Integration (reported only in Discussion)	Interviews during each remaining trimester of preg, asking about maternal Cnb, nicotine, caffeine, and alcohol use; nutrition; sociodemographic characteristics; health Subsequent interviews assessed postnatal exposure to Cnb smoke and other potential covariates	# joints/wk Categories: Heavy use (≥6 joints/wk) Infrequent/ moderate (>0 and <6 joints/wk) Nonuser Mean Cnb use for infrequent/ moderate and heavy users was 1.9 joints/wk and 16.1 joints/wk, respectively (used in trend analyses)	<u>Discriminant Function Analyses</u> Prenatal Cnb use assoc w/ aspects of <u>executive function</u> Main discriminating measures on which Cnb-exposed children performed more poorly than unexposed (structure coefficients): Category Test (0.49) Gordon Delay total responses (0.34) WISC-Block Design (-0.30) WISC-Picture Completion (-0.30) Gordon Delay efficiency ratio (-0.29) <u>Trend Analyses</u> Mean (SE) scores by prenatal Cnb use, adjusted for pre- and postnatal confounders: WISC Comprehension nonuser 10.7 (0.35) moderate 12.6 (0.78) heavy 11.9 (0.90) **p≤0.05 for quadratic trend** WISC Block Design nonuser 12.3 (0.36) moderate 12.3 (0.86) heavy 10.5 (0.96) p≤0.10 for linear trend WISC Object Assembly nonuser 11.2 (0.29) moderate 12.7 (0.67) heavy 10.4 (0.72) **p≤0.05 for quadratic trend**	<u>Included in DFA</u> Maternal age at delivery Prenatal nicotine use Prenatal alcohol use Current maternal Cnb use Variables included in trend analyses not specified <u>Considered</u> *<u>Prenatal</u>* Family status Family income Parent education Prenatal passive smoke exposure *<u>Postnatal</u>* Child's sex Home Environment Questionnaire (10 scales) Mother's personality (NEO 5 Factor Inventory) Family income Maternal education	No differential loss of subjects with respect to drug use variables Additional questionnaires used to examine children's anxiety and depression Authors' discussion: WISC-Block Design subtest requires perceptual organization, spatial visualization, and abstract conceptualization. WISC-Picture Completion subtest assesses ability to differentiate essential from nonessential details. Both potentially involve visuo-spatial and visuo-motor abilities, and higher-order cognitive processes, such as planning, impulse control, visuo-construction and visuo-analysis. To test basic spatial and visual motor functioning, the Developmental Test of Visual-Motor Integration was administered. The test was not assoc with prenatal Cnb exposure, and inclusion as a covariate did not diminish assoc btwn Cnb use and discriminant outcome scores, suggesting maternal Cnb use does not affect basic visual and motor functioning, but

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	administered without knowledge of prenatal drug exposure ANCOVA, Discriminant function analysis (DFA)				Gordon Vigilance (number correct) nonuser 91.9 (2.3) moderate 98.4 (5.3) heavy 107.8 (5.6) **p≤0.05 for linear trend** Gordon Vigilance Commissions (errors) nonuser 116.5 (2.7) moderate 112.3 (6.3) heavy 98.5 (6.8) **p≤0.05 for linear trend** Category Test Total Errors nonuser 86.5 (2.0) moderate 90.7 (4.4) heavy 96.4 (5.1) p≤0.10 for linear trend No assoc with Full Scale IQ or composite Verbal IQ		rather “higher order” cognitive processes. Authors also note that WISC-Block design and Picture Completion require visual analysis, hypothesis testing, and inhibition of prepotent responses, which is consistent with the Category Test, Gordon Delay Task, and Gordon Efficiency Ratio (correct responses/total responses). <u>Strengths</u> Discriminant function analysis was cross-validated

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Golds-chmidt et al. 2000 Pittsburgh, PA MHPCD	Prospective cohort N = 1360 Participants were recruited from women ≥18 yo, attending 4-mo prenatal visit at Magee-Womens Hospital prenatal clinic 1982 – 1985 Combined 2 cohorts: 1) all women who used 2+ Cnb joints/mo during the 1st trimester, 2) all women who drank alcohol 3+ times/wk, and a random sample of women who used less than this amount of Cnb or alcohol Birth cohort included 763 live singletons. Attrition was due mainly to: moved out of state, refused to participate, loss to follow-up Final cohort for 10-yr follow up study, conducted 1994-1997: n = 635 mother's reports	Child behavior problems at 10 yo, assessed w/: Swanson, Nolan, and Pelham (SNAP) questionnaire, completed by mothers. Elicits symptoms of attention deficit disorder with hyperactivity with 4 subscales: hyperactivity, inattention, impulsivity, peer problems Child Behavior Checklist (CBCL), completed by mothers. Assesses internalizing traits (withdrawn, somatic complaints, anxious/ depressed) and externalizing traits (delinquent behavior and aggressive behavior), among others Teacher's Report Form (TRF) is similar to the CBCL Borderline clinical cutoff score for CBCL and TRF subscales was 67	Women were interviewed about 1st, 2nd, and 3rd trimester substance use in their 4th and 7th mo prenatal visit and after delivery, respectively Women and children were assessed at birth, 8 and 18 mo, and 3, 6, and 10 yrs of age. At each phase, mothers were asked about substance use, social and psychological status, and environment of the child Women were asked about amount and frequency of Cnb, hashish, and sinsemilla use. A bowl/joint of hashish was counted as 3 joints of Cnb, and sinsemilla as 2 joints	Average daily joints (ADJ), analyzed as continuous and categorical variables 1st trimester Cnb categories: Nonusers Light to moderate users 0<ADJ≤0.89 Heavy users ADJ>0.89 2nd and 3rd trimester Cnb categories: Nonusers Light users 0<ADJ≤0.4 Moderate-heavy users ADJ>0.4 0.89 ADJ is about 1 joint/day	Prenatal Cnb use was significantly related to increased hyperactivity, impulsivity, and inattention symptoms (measured by SNAP), increased delinquency (measured by CBCL), and increased delinquency/externalizing problems (measured by TRF) SNAP: 3rd trimester Cnb use was assoc w/ higher scores on hyperactivity (**β=1.2, p<0.001**) inattention (**β=0.9, p<0.01**), and impulsivity (**β=1.0, p<0.01**); no dose-response relationship Regression coefficients for analysis with 3rd trimester Cnb exposure dichotomized to compare moderate and heavy users to none and light users: hyperactivity (**β=1.2, p<0.01**) inattention (**β=1.3, p<0.001**) impulsivity (**β=1.2, p<0.01**) Avg hyperactivity scale scores by 3rd trimester Cnb use: Nonuser 9.6 Light to moderate user 9.6 Heavy user 10.9 [**F(2,632)=4.7, p=0.01**] Avg inattention scale scores by 3rd trimester Cnb use: Nonuser 8.8 Light to moderate user 8.7 Heavy user 10.2 [**F(2,632)=5.4, p=0.005**] Avg impulsivity scale scores by 3rd trimester Cnb use: Nonuser 10.1 Light to moderate user 9.9	For all analyses, variables that were ss at α level of 0.05 were retained in models Maternal education Maternal work/ school status Family income Male in household Ethnicity Maternal psychosocial characteristics Home environment No. of siblings Maternal child custody Child's age Child's gender Child's illnesses, injuries, hospitalizations Prenatal alcohol, tobacco, cocaine use Current maternal alcohol, tobacco, cocaine use ADHD medication	Mothers were low-income, 47% Caucasian, 53% African-American, low SES No ss differences in prenatal substance use btwn women who participated and those who were eligible but did not participate at 10 yrs No ss differences in prenatal or current substance use, current environment, or maternal behavior ratings btwn children w/ and w/o a completed TRF Strengths: Thorough exposure assessment Consideration of many potential confounders Limitations: Multiple comparisons

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	n = 575 teacher's reports Multiple regression, logistic regression, ANOVA, chi-squared, structural equation modeling				Heavy user 11.4 [**F(2,632)=4.4, p=0.01**] 1st trimester Cnb use was assoc w/ the inattention scale of SNAP (**β=0.7, p<0.01**) Avg scores by Cnb use: Nonuser 8.6 Light to moderate user 9.1 Heavy user 9.7 [**F(2,632)=5.4, p=0.005**] Cnb use was ns assoc w/ any other SNAP subscales CBCL: 2nd trimester Cnb use assoc w/ fewer internalizing behavior problems (**β=-3.0, p<0.05**) 1st trimester heavy Cnb use compared to non-heavy use assoc w/ scoring above the borderline clinical cutoff of the delinquency subscale, **RR= 2.4 (1.3, 4.5), p<0.01**. Cnb use was ns assoc w/ any other CBCL subscales Path model: Structural equation model to test hypothesis that inattention symptoms mediate expression of delinquency. 1st trimester Cnb exposure was directly related to increased inattention symptoms (SNAP) at age 10 and indirectly related to delinquency (CBCL) through inattention symptoms. Chi-squared goodness of fit test statistic was 9.5, indicating "perfect data-model fit with 38 degrees of freedom" TRF: Avg externalizing scores by 2nd trimester Cnb use: Nonuser 53.4	Aggression among family members	

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					Light user 53.6 Moderate/heavy user 58.7 [**F(2,517)=4.3, p=0.01**] Avg externalizing scores by 3rd trimester Cnb use: Nonuser 53.6 Light user 54.6 Moderate/heavy user 59.2 [**F(2,572)=5.2, p=0.006**] When dichotomized into abstainers/light users and moderate/heavy users, 2nd trimester use was assoc w/ externalizing score (**β=4.3, p<0.01**) and total behavior problem score (**β=3.9, p<0.05**) Dichotomized 3rd trimester Cnb use was also assoc w/ TRF externalizing score (**β=4.3, p=0.006**) These results indicate that Cnb use above a threshold of >3 joints/wk in 2nd & 3rd trimesters predicted an increased rate of externalizing problems, as reported by teachers on the TRF Percent of TRF delinquency scores above the borderline cutpoint, by 2nd trimester Cnb: Nonuser 11 Light user 10 Moderate/heavy user 23, **p<0.05** Percent of TRF delinquency scores above the borderline cutpoint, by 3rd trimester Cnb use: Nonuser 12 Light user 5 Moderate/heavy user 27, **p<0.01** RRs of scoring above clinical cutoff for TRF delinquency for		

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					moderate/heavy use vs. abstainers/light users: 2nd trimester: **2.3 (1.02, 5.05), p=0.04** 3rd trimester: **3.0 (1.4, 6.4), p<0.01** Cnb use was ns for any other TRF subscales Path analysis: Effects of 3rd trimester exposure on delinquency were mediated by effects of exposure on attention. 'The structural equation model fit the data perfectly: chi-squared goodness of fit = 3.6, 38 degrees of freedom'		

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Gold-schmidt et al. 2012 Pittsburgh, PA MHPCD	Prospective cohort Participants were recruited from prenatal clinic at Magee-Womens Hospital 1982 – 1985 N = 763 live singleton births Cohort included all women who used ≥2 Cnb joints/mo in the 1st trimester and a random sample of women who used less than this amount, and women who drank ≥3 drinks per week during the 1st trimester and a random sample of women who used less than this amount were selected. n = 524 mother-child dyads seen at 14 years Attrition due mainly to loss to follow-up, refusal, moved out of the area, or child was interviewed by phone and could not be tested. Multiple regression, structural equation models	<u>Primary Outcome</u>: School achievement at 14 yrs, measured by Wechsler Individual Achievement Test (WIAT). WIAT Screener has 3 subtests: basic reading, mathematics reasoning, spelling. Age-adjusted composite and subtests scores were used <u>Potential Mediators</u>: Child's cognitive development at 6 yrs, measured by the Stanford-Binet Intelligence Scale (SBIS) Childhood depressive symptoms at 10 yrs, measured by Children's Depression Inventory (CDI) Attention problems at 10 yrs, measured by the Swanson, Noland, and Pelham (SNAP) attention subscale Adolescent substance use, measured through questions adapted from Health Behavior Questionnaire	Maternal self-report Women were interviewed about 1st, 2nd, and 3rd trimester substance use in their 4th or 5th and 7th mo prenatal visit and after delivery, respectively. Women and their offspring were assessed at birth, 8 and 18 mo, and 3, 6, 10, 14, 16, and 22 yrs of age. At each phase, maternal interviews included questions about substance use, sociodemograph-ic and environmental characteristics, and psychological status	Average daily joints (ADJ) calculated from assessments of quantity and frequency of Cnb use across all three trimesters Categories: Heavy users: ADJ ≥ 1 Non-heavy: ADJ<1 Sinsemilla was counted as 2 joints of Cnb and hashish as 3 joints of Cnb	<u>Regression results</u> 1st trimester heavy Cnb exposure assoc w/ lower WIAT composite **(-2.9 points, p<0.05)** and basic reading **(-3.3 points, p<0.05)** scores <u>Structural equation models</u> Deficit in achievement was mediated by effects of prenatal Cnb use on SBIS (intelligence) at 6 yrs, SNAP (attention problems) and CDI (depression) at 10 yrs, and initiation of Cnb use before age 14. Direct effects of 1st trimester Cnb exposure on WIAT score at 14 yrs, before addition of intervening mediators (standardized coefficient): 1st trimester Cnb → WIAT score (**-0.08, p<0.05**) Direct effects of 1st trimester Cnb on WIAT score at 14 yrs, after addition of 4 mediators (standardized coefficient): 1st trimester Cnb → WIAT score (-0.01, p>0.05) Total effects of 1st trimester Cnb exposure on WIAT score at 14 yrs, w/ mediators: 1st trimester Cnb → SNAP (standardized coefficient for assoc btwn Cnb and SNAP: **0.09, p<0.05**) → WIAT (standardized coefficient for	Covariates were selected from the following, based on assoc w/ school achievement in literature and whether ss in model Prenatal alcohol, tobacco, and other substance use Offspring gender and ethnicity Home environment Maternal SES Current maternal substance use Presence of an adult male in household Adolescent in maternal custody No. of siblings Maternal depression and hostility Maternal no. of life events Social support for mother Overall coping	The direct effects of prenatal Cnb on school achievement was ss only when mediators were not taken into account. After including IQ, attention problems, depressive symptoms, and early age of Cnb initiation, the effect was ns. The authors postulate that effects of prenatal Cnb on academic achievement is mediated by its earlier effects on these mediating factors In regression analyses, the lowest exposure group was exposed to <1 ADJ No differences in gender, BW, maternal income, or maternal education btwn participants and non-participants. Participants were more likely to be African-American and have 3rd trimester Cnb exposure Half the adolescent sample is male and 55% are African American <u>Strengths:</u> Prospective design Sample size and high retention rates increased statistical power <u>Limitations:</u> Sample consisted largely

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					assoc btwn SNAP and WIAT: **-0.11, p<0.005**) 1st trimester Cnb → adolescent Cnb use before 14 **(0.08, p<0.05)** → WIAT **(-0.05, p<0.05)** 1st trimester Cnb → CDI **(0.18 p<0.0005)** → WIAT → **(-0.09 p<0.05)** 1st trimester Cnb→ SBIS composite score **(-0.08, p<0.05)** → WIAT **(0.53 p<0.0005)** 1st trimester Cnb → SBIS composite score **(-0.08, p<0.05)** → CDI **(-0.16 p<0.0005)** → WIAT **(-0.09 p<0.05)** 85% of the effect of prenatal Cnb use on WIAT composite score was due to mediators Total effects of prenatal Cnb on WIAT *reading* score (standardized coefficient): **-0.1 (p<0.05);** 60% of effect was explained by mediators Mediators of WIAT reading score (standardized coefficients) SBIS (0.45, p<0.0005) SNAP (-0.11, p<0.005) CDI (-0.06, p<0.1) Cnb use before 14 (-0.06, p<0.01)	ability of mother	of low SES women, possibly limiting generalizability

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					Indirect effects of prenatal Cnb on WIAT reading score through mediators all ss Direct effect of prenatal Cnb on WIAT reading score ns after inclusion of mediators 2nd and 3rd trimester Cnb exposure ns assoc w/ WIAT No assoc btwn WIAT and other prenatal substance exposures		

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Leech et al. 1999 Pittsburgh, PA MHPCD	Prospective cohort N = 1360 Participants recruited from prenatal clinic at Magee-Womens Hospital from 1983 - 1985 Women were ≥18 yo 2 cohorts selected, (women could be in 1 or both cohorts, alcohol and Cnb) were sampled with replacement Eligibility criteria: - women who drank ≥3 alcoholic drinks/wk in the 1st trimester and a random sample of 1/3 of women who drank ≤3 drinks; - women who used Cnb ≥2 joints per mo in the 1st trimester, and a random sample of 1/3 of women who used ≤2 times Both cohorts were combined for these analyses Participants were not included due to: Family moved >150	Attention and impulsivity in 6 yo offspring examined using a Continuous Performance Task (CPT) Study focused on prenatal exposure to alcohol, tobacco, Cnb and cocaine Errors of commission were indicative of impulsive responding Errors of omission were indicative of inattentiveness	Maternal self-report Women were interviewed at 4th and 7th mo prenatal visit and at delivery Additional follow-up interviews and assessments occurred at 8 and 18 mos, and 3, 6, 10 yrs postpartum At each follow-up, mothers were interviewed with a standardized instrument that measured alcohol, Cnb, tobacco, cocaine, and other illicit drug use. Additional information about psychological, social, and environmental factors, demographic status, and medical history was also collected. At each phase,	Interview assessed usual, maximum, and minimum frequency and quantity of Cnb, hashish, and sinsemilla 1 hashish joint or bowl = 3 Cnb joints 1 sinsemilla = 2 Cnb joints Cnb use summarized as average daily joints (ADJ) Mothers' prenatal Cnb use (≥1 joint/day): 1st trimester =15%; 2nd and 3rd trimester = 5%	Stepwise regression assessing assoc btwn prenatal Cnb exposure by trimester and errors of commission and omission: unstandardized β, standardized 1st trimester: Errors of commission: 0.10, 0.02 Errors of omission: -0.09, -0.03 2nd trimester: Errors of commission: **1.21 p<0.01**, 0.13 Errors of omission: **-0.56 p<0.05**, -0.10 3rd trimester: Errors of commission: 0.36, 0.06 Errors of omission: -0.34, -0.07 There were ns effects of prenatal alcohol, cocaine, or tobacco exposure on errors of commission for any trimester of preg Tobacco use during the 2nd and 3rd trimesters significantly predicted more errors of omission. Cocaine exposure in the 1st trimester was a ss predictor of increased errors	<u>Included:</u> Child's age at assessment Composite Stanford-Binet score Gender No. of child hospital-izations No. of child illnesses Child's race Home screening question-naire Male in household Maternal work/school status Maternal hostility Maternal life events Maternal age Current maternal use of Cnb, alcohol, and cocaine at 6 yrs Prenatal maternal use of Cnb (trimester	This study found prenatal Cnb exposure to increase errors in commission indicating more impulsivity. Results also showed Cnb exposed offspring made fewer errors of omission-indicating positive effects on attention. Women were "light to moderate users of alcohol, Cnb and cocaine". Use of tobacco was high. Women were mostly African-American and of lower SES. <u>Strengths:</u> Assessed and controlled for potential influences such as postnatal substance exposure and characteristics of the child. Used hierarchical modeling to determine the influence of other factors such as maternal characteristics relative to prenatal Cnb exposure. <u>Limitations:</u> Authors note that the CPT used in this study required a response to a single stimulus and therefore may not have been

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	miles away The child's whereabouts were untraceable due to placement The child did not complete the Continuous performance task [CPT] (due to impulse control or attention problems) Handicap (4) Colorblindness n = 608 Stepwise multiple regression, Cook's statistic, hierarchical regression		central nervous system (CNS) development was evaluated, and growth and morphologic abnormalities were assessed		of omission (all were unstandardized) There was no assoc w/ errors of omission and prenatal alcohol exposure	specific), alcohol, tobacco, and cocaine <u>Considered:</u> Child's attendance of special education class Child's grade in school Child's hearing or vision problems Child's no. of injuries Family income Marital status Maternal education No. of siblings Preg, labor or delivery complic-ations Maternal depression Current maternal tobacco use at 6 yo	difficult enough. Additionally, this CPT did not allow for comparison btwn different types of commission errors. Authors also state the lack of a measure of reaction time as a limitation. It was not possible to separate the effects of prenatal exposure from current environmental exposure at 6 yo due to the high correlations btwn the two.

Study/ Location	*Study Design, Sample Sizes, Statistical Analysis*	*Outcomes of Interest*	*Exposure Measurement Methods*	*Exposure quantification*	*Results*	*Covariates/ Confounders*	*Comments*
Noland et al. 2005 Cleveland, OH	Prospective cohort N = 415 Participants had been followed since birth. Mothers were recruited and screened for drug use at a large, urban county hospital (Singer et al. 1999) 376 children from the original cohort were alive and available at 4 yrs. 46 were not given the attention tasks due to the researchers' readiness or availability, or because: child's IQ<45, home visit was not possible, or the family left and did not return n = 330 children were offered at least 1 of 2 attention tasks: continuous performance task (CPT) or picture deletion task (PDT) 149 children were not offered the CPT due to inadequate time or personnel, CPT was still being piloted, or child's IQ≤70	Selective attention and sustained attention Preschoolers/ 4 yo Assessed by CPT and PDT Both tests were modified for this cohort 301 of 329 children who were offered the PDT, and 154 of 181 children offered the CPT passed the pre-test and went on to participate in the tests	Meconium analysis Infant/Maternal urine analysis Standardized interview at 2 wks post-partum	Assignment to Cnb-exposed group required a positive response to maternal self-report, meconium analysis, or infant/maternal urine analysis Severity of Cnb use: days/wk of use multiplied by daily no. of joints for the mo prior to preg and for each trimester of preg Four severity scores were averaged into a single avg severity score	Avg severity of Cnb exposure was marginally correlated w/ the rate of omission errors on PDT (r=0.11, p<0.08), suggesting prenatal Cnb exposure is assoc w/ worse sustained attention Adjusted for prenatal cocaine exposure, severity of 1st trimester Cnb exposure was related to more omission errors (**β= 0.32, p = 0.03)**. Further adjusting for severity of caregiver current Cnb use reduced the assoc btwn 1st trimester Cnb use and PDT omission errors (β= 0.29, p = 0.06)	<u>Included</u> 1st trimester cocaine exposure, severity Caregiver current Cnb use, severity <u>Considered:</u> Gender African-American (mother) Maternal age at birth Parity Prenatal care Maternal education Marital status Low SES Biological and current caregiver mental functioning and substance use variables <u>Considered as mediators</u> GA BL BW HC Child current verbal IQ	The study finds that at 4 yrs of age Cnb exposed children have some difficulty w/ sustained attention. <u>Strengths:</u> Multiple exposure methods Assessed birth mother and current caregiver characteristics as covariates <u>Limitations</u>: Majority of sample African-American and low SES, therefore results may not be generalizable to other populations.

Study/ Location	Study Design, Sample Sizes, Statistical Analysis	Outcomes of Interest	Exposure Measurement Methods	Exposure quantification	Results	Covariates/ Confounders	Comments
	Testers were blinded to exposure status Current caregivers were also assessed for psychiatric symptoms, verbal ability, and IQ at the postpartum assessment. At the 4 yo visit a version of the HOME assessment was verbally administered, drug use interview updated, and assessments from the postpartum visit were repeated if the caregiver was new T-test, Pearson correlations, generalized additive models, logistic regression						

Study/ Location	*Study Design, Sample Sizes, Statistical Analysis*	*Outcomes of Interest*	*Exposure Measurement Methods*	*Exposure quantification*	*Results*	*Covariates/ Confounders*	*Comments*
O'Connell and Fried 1991 Ottawa, Canada OPPS	Prospective cohort N not stated Participants were recruited through notices in obstetricians offices and public media and interviewed about prescription and other drug use, health history, and diet All 6-9 yo children whose mothers consumed >1 Cnb joint/wk during preg were selected (n exposed = 28) Children in control group (no maternal use of Cnb in preg) were matched w/ exposed children based on maternal tobacco and alcohol use during preg (n unexposed = 28) n = 56 The same examiner conducted the child assessments and was blind to maternal drug history Discriminant function analysis, path analysis, multivariate analysis of	Neurobehavioral development in 6-9 yo offspring, incl. ratings of behavior problems, visual-perceptual tasks, language comprehension, and distractibility Test battery included: Global intellectual measures (WISC-R) Test of Visual Perceptual Skills Visual-motor integration (Test of Visual Motor Integration, Draw-A-Man, and Trail Making Test) Attention and memory tasks (Stroop Color	Maternal self-report	Regular Cnb use (avg >1 joint/wk during preg) was compared with no Cnb use during preg Exposure ranged from 1.5 to 50 joints/wk among Cnb users	Discriminant function analysis: 7 outcome variables discriminated between the Cnb users and nonusers, explaining 36% of the group variance: Syntax Quotient Stroop Interference Visual Discrimination Visual Sequential Memory Trail Making Test-Part A Anxiety Conduct problems Effect of maternal Cnb use was evident in increased conduct problems (**F=7.39, p=0.01)** and to a lesser degree, poorer visual sequential memory (F=3.13, p=0.08) and poorer visual discrimination (F=2.89, p=0.09) After controlling for covariates, interaction of Cnb use and maternal age was assoc w/ outcomes (p=0.01), principally visual sequential memory (F(1,46)=8.69, p=0.01) and syntax quotient (F(1,46)=4.11, p=0.05): offspring of younger Cnb users scored lowest and children of younger control women scored highest on tests. The effect of Cnb use in	Included: Aggression-external Aggression in the home Supervision Agreeableness Conscientious-ness Neuroticism Mother's age at delivery Considered: Location of test HOME questionnaire variables Maternal and, paternal education Income Child's health School progress Family size Birth order Principal languages at home and school Mother's personality and intelligence Other sociodemo-graphic information	Authors note that univariate trends in this study are consistent with previous reports on young children, e.g., language comprehension scores, memory test scores, and ratings of child behavior were lower for children of Cnb users Strengths: Authors calculated 84% statistical power based on an effect size of 0.4 standard deviations Limitations: Sample size too small to permit extensive matching of subjects on potentially important confounders Authors note that the main findings were related to parental rating of child behavior problems. Whether these findings indicate true behavioral differences in children or different levels of parental tolerance is unclear. Postnatal Cnb exposure was not assessed in this study 6 women in the Cnb user group and none of the non-users reported use of

Study/ Location	Study Design, Sample Sizes, Statistical Analysis	Outcomes of Interest	Exposure Measurement Methods	Exposure quantification	Results	Covariates/ Confounders	Comments
	covariance, multiple regression	and Word Test, Knox Cube Test) Motoric assessment (Finger Tapping Test) Language comprehen-sion (Syntax Quotient derived from the Test of Language Development-Primary) Academic achievement (3 subtests of the Wide Range Achievement Test-Revised, Passage Comprehen_ sion subtest of the Woodcock Reading Mastery Test) Parent ratings of behavior problems (Conners Parent Rating Scale)			this model was diminished (p=0.11). When the 6 mothers who reported other illicit drug use were excluded from analysis, the main effect of Cnb use on discriminating outcome variables was ss **(p=0.02),** as was the interaction btwn Cnb use and maternal age at delivery **(p=0.05)** Path analysis: In analysis of conduct problems, of the 6 covariates, only aggression in the home was ss (Beta weight **B=0.51, p=0.02)**. Including aggression in the home with maternal Cnb use results in a ss model (**R^2=0.28, p=0.0002)**. The combined indirect and direct effect of maternal Cnb use on conduct problems was 0.43, which approximated the zero-order correlation btwn the two variables (r=.39). No dose-response relationship was detected		other drugs in preg (all infrequent, mostly cocaine).

Study/ Location	*Study Design, Sample Sizes, Statistical Analysis*	*Outcomes of Interest*	*Exposure Measurement Methods*	*Exposure quantification*	*Results*	*Covariates/ Confounders*	*Comments*
Richardson et al. 2002 Pittsburgh, PA MHPCD	Prospective cohort 10 yr follow up conducted btwn 1994-1997 N = 1360 women interviewed at 4th or 5th mo of preg 2 cohorts combined: (1) All women who drank ≥ 3 alcoholic drinks/wk and a random sample of women who drank less, (2) all women who smoked ≥ 2 marijuana (Cnb) joints/mo and a random sample who used less Cnb (women could be in both cohorts) n = 763 Eligibility criteria: live, singleton birth, available for 10-yr follow up, no condition that interfered w/ testing n = 636 (91% of eligible participants; 83% of birth cohort) n = 593 (after excluding children w/ conditions	Cognitive domains at 10 yrs of age (assessment tool) Problem solving and abstract reasoning, as well as perseverative reasoning and impulsivity (Wisconsin Card Sorting Test) Learning and memory (Wide Range Assessment of Memory and Learning (WRAML)) Mental flexibility, attention, visuomotor tracking, problem solving (Trail Making, Parts A and B, and Stroop Test) Psychomotor speed and eye-hand coordination (Grooved Pegboard) Attention and general mental efficiency, including impulsivity (Continuous Performance Test (CPT-2)) The following tests were also used: HOME-SF assessed the quality of cognitive stimulation in the home and emotional support Stanford-Binet Intelligence Scale-4th Edition assessed intellectual development Revised Children's Manifest Anxiety Scale	Maternal self-report Women were interviewed at 4th and 7th mo prenatal visit and at delivery Additional follow-up interviews and assessments occurred at 8 and 18 mos, and 3, 6, 10 yrs postpartum Women were asked about maximum, minimum, and usual amount and frequency of Cnb, hashish, and sinsemilla use Regression analyses were run separately for Cnb in each trimester and for different measures of Cnb	Interview assessed usual, maximum, and minimum frequency and quantity of Cnb, hashish, and sinsemilla Based on amount of THC in each substance: 1 hashish joint or bowl = 3 Cnb joints 1 sinsemilla = 2 Cnb joints Cnb use summarized as average daily joints (ADJ) Mothers' prenatal Cnb use (≥1 joint/day): 1st trimester =15%; 2nd and 3rd trimester = 5% *Cnb use categories: a*vg no. of joints/day (ADJ) None: no use, Light: 0<ADJ≤0.4 joints/day; Moderate: (0.4<ADJ≤ 0.89 joints/day; Heavy:	<u>Learning and memory (WRAML)</u>: 1st trimester heavy Cnb use ss assoc w/ lower scores on design memory **(β=−0.83)** and screening index (**β=−3.34**) Alcohol and tobacco use also were ss predictors for these subscales Attention and general mental efficiency <u>(CPT-2)</u>: 2nd trimester ADJ predicted ss more Trial 3 commission errors, a measure of impulsivity (**β=1.86**) (errors of commission appeared only toward the end of the task when the children were fatigued) Problem solving and abstract reasoning, mental flexibility, psychomotor speed and eye-hand coordination were ns assoc w/ Cnb use in any trimester The regression analyses showed that average daily alcohol use and frequency of drinking four or more drinks/day both predicted poorer performance on verbal learning and verbal and visual memory tasks Alcohol and tobacco use was also observed to affect learning and memory	<u>Included</u> Education Family income Race Child's age* Child's anxiety Child's gender HOME-SF Child's uncorrected vision problems Prenatal alcohol and tobacco use <u>Considered</u> Maternal age Male presence Work/school status Maternal depression Maternal IQ Maternal hostility Maternal life events Child's hospitalizations, illnesses, injuries No. of siblings Child's Stanford-Binet Intelligence	Children who did not participate at 10 yrs were older and had fewer illnesses, and mothers who did not participate used less alcohol at 10 yrs and less Cnb in the 1st trimester. Twenty-three percent of children had repeated one or more grades. Stanford-Binet Intelligence scale composite score was 91.5 (range = 59-130). Authors stated they found that prenatal alcohol and Cnb exposure specifically affected learning and memory. Findings for prenatal Cnb exposure and increased impulsivity were consistent w/ results seen at 6 yrs. Prenatal tobacco use was also found to affect learning and memory, consistent w/ an earlier report from this project. Authors stated that the findings are particularly notable as the cohort represents children who have been exposed to light to moderate levels of drugs. <u>Strengths:</u> Detailed assessment of

Study/ Location	Study Design, Sample Sizes, Statistical Analysis	Outcomes of Interest	Exposure Measurement Methods	Exposure quantification	Results	Covariates/ Confounders	Comments
	that would interfere w/ testing) Structural equation model, t test, multiple regression Structural equation modeling was used to identify the assoc btwn exposures and neuropyschological domains	assessed anxiety-related symptoms		ADJ>0.89joints /day Prevalence of use in 1st, 2nd, 3rd trimesters, and next 10 yrs by use category None: 58, 77, 81, 78 Light use: 19, 14, 11, 17 Moderate use: 8, 4, 3, 2 Heavy use: 14, 5, 5, 3 The majority of women reduced their Cnb use as preg progressed Dichotomous variable was constructed to examine the effect of heavy use (<0.89 vs ≥0.89 joints/day		Scale Current maternal use of alcohol, cocaine, and tobacco Prenatal cocaine * Not included in WRAML model	Cnb use, as well alcohol,, tobacco, and other drug use during preg (by trimester) and at each follow-up phase.

Study/ Location	***Study Design, Sample Sizes, Statistical Analysis***	***Outcomes of Interest***	***Exposure Measurement Methods***	***Exposure quantification***	***Results***	***Covariates/ Confounders***	***Comments***
Rose-Jacobs et al. 2017 Boston, MA Boston City Hospital	Original cohort recruited mothers from Boston City Hospital 8-72 hours after 1990 - 1993 Participants (offspring) were high school students n = 131 <u>Eligibility Criteria:</u> GA≥ 36 wks No level III NICU care No diagnosed FAS No diagnosed HIV Mother's English fluency Mother ≥ 18 yo No documented use of illicit substances other than Cnb and cocaine No PTB No genetic syndromes Generalized estimating equation models, multivariable linear models	Executive Functioning (EF) as assessed by the Behavior Rating Inventory of Executive Functioning-Teacher Form (BRIEF-TF) every year Authors used behavioral regulation and metacognition as the primary indexes Higher scores indicate less optimal functioning Behavioral regulation refers to the ability to shift attention btwn different tasks, adaptability, and ability to regulate strong/automatic emotional responses Metacognition scores indicate higher order thinking such as the ability to understand, analyze and control one's cognitive processes in order to manage performance	<u>Intrauterine exposure:</u> Urine samples analyzed for Cnb Meconium samples analyzed for Cnb Maternal self-report Urine samples were analyzed using radio-immunoassay for benzoylecgonine, opiates, amphetamines, benzodiazepines and markers of Cnb (cannabinoids) Meconium specimens were analyzed by radio-immunoassay for the cocaine metabolite benzoylecgonine, metabolites of opiates, amphetamines, benzodiazepines, and markers of Cnb (cannabinoids)	<u>Intrauterine substance exposure classification:</u> Intrauterine marijuana exposure (IUME) based on a composite of urine assays, meconium assays, and maternal self-report Exposure was categorized as "unexposed" (n=97), "lighter" (n=19), "heavier" (n=15) Adolescent were considered substance-positive "if they self-reported use of alcohol, Cnb, and other substances excluding tobacco experimentation during the	Assoc btwn IUME and Behavioral regulation T-score as measured by multivariable linear models: β (95% CI) Heavier vs unexposed: **-11.6 (-20.8, -2.48) p=0.01** (indicating heavier use was ss assoc w/ more optimal Behavioral Regulation) Lighter vs. unexposed: -3.64 (-10.9, 3.68) p=0.33 Assoc btwn IUME and Metacognition T-Score as measured by multivariable linear models: β (95% CI) Heavier vs unexposed: **-11.9 (-19.91, -3.93) p=0.003** Lighter vs. unexposed: -2.32 (-8.39, 3.75) p=0.45 Authors also state that heavier IUME vs no exposure was assoc w/ significantly less metacognition clinical risk, **OR = 0.3, p=0.04** IUTE was ss assoc w/ less optimal EF (BRIEF-TF Behavioral Regulations scores **(p<0.05)**	<u>Included:</u> Each of the other intrauterine substance exposures Maternal age Maternal race Maternal education Maternal nation of birth Child's sex BW z-score for GA Adolescent age at time of BRIEF-TF assessment Highest level of lead exposure up to 4 yo Adolescent IQ Exposure to violence <u>Considered:</u> Childhood exposure to second-hand smoke Adolescent's own substance use Any adolescent problematic substance use Primiparous vs	The cohort was recruited from an urban hospital serving a large Medicaid population. (89% African American/ Caribbean). IUME was independently assoc w/ more optimal behavior regulation and metacognition scores. This was in contrast to author's expectations and some prior studies. Authors state that explanations or mechanisms for these results "remain uncertain". Focus of the study was tobacco exposure. <u>Strengths:</u> Multiple exposure measurement methods. Substance use was determined, by both self-report and biologic measures, and analyzed for prenatal and adolescent exposure. The study was able to isolate the assoc w/ each substance independently. <u>Limitations:</u> Authors note that because of the subjective nature of the BRIEF-TF

Study/ Location	Study Design, Sample Sizes, Statistical Analysis	Outcomes of Interest	Exposure Measurement Methods	Exposure quantification	Results	Covariates/ Confounders	Comments
			Exposure in: Adolescents: adolescents voluntarily provided urine samples at each visit to screen for legal and illegal substances No-Excuse Urine test used to analyze for cannabanoids "gives a detection window of up to a wk for most drugs, and even longer for marijuana"	past 30 days or if their urine assay was positive" Adolescents reporting substance use prior to the past 30 days and w/ a negative urine assay were considered "ever" having used a particular substance		other Neonatal HC Household tobacco use Number of caregiver changes prior to late adolescence	assessment, racial bias may affect the predominantly African-American sample. Adolescents that dropped out of high school may have had less optimal scores but could not be included in the analysis. The focus of the paper was prenatal exposure to tobacco smoke. The number of adolescents prenatally exposed to Cnb was small.

Study/ Location	***Study Design, Sample Sizes, Statistical Analysis***	***Outcomes of Interest***	***Exposure Measurement Methods***	***Exposure quantification***	***Results***	***Covariates/ Confounders***	***Comments***
Smith et al. (2004) Ottawa, Canada OPPS Same population as in Smith et al. (2006) and 2016	Prospective cohort Preg women were recruited from 1979 - 1983 Participants were randomly selected from OPPS offspring <u>Eligibility criteria</u>: Completed the WAIS-III and C-DISC Clinical Test w/in 1 yr prior to the study 18-22 yo Right handed No Axis I DSM-IV diagnosis No structural abnormalities English as the first language n = 35 4 participants were excluded due to current cocaine, opiates or amphetamine use (as per urine test, or self-reported use defined as ≥1x/mo)	Response inhibition Objective was to examine assoc btwn prenatal Cnb exposure and prefrontal cortex activity in young adults Participants engaged in a "Go/No-Go" task (which requires participant to make a motor response to a particular stimulus (press for x) and to withhold responding to a different stimulus (press for all letters except x)) Testing was performed while functional magnetic resonance imaging (fMRI) was conducted to assess brain activity	Maternal self-report through interviews conducted in woman's home for each trimester left after enrolling in study (as detailed in Fried et al. 1980; Fried and O'Connell et al. 1987)	Prenatal Cnb exposure defined as regular maternal use of at least 1 joint/wk for the entire preg n = 15 non-exposed n = 16 exposed (joints/wk): Range=0.33-54 Mean (SE) = 8.27 (3.24) Exposure was analyzed as a continuous variable	*Behavioral differences:* "As expected, no statistical differences in errors of omission or reaction time were found btwn Cnb exposed and unexposed offspring Cnb exposed group had ss more errors of commission (Press for all letters except x) Mean (SE) exposed vs non-exposed 5.56 (1.05) vs 2.8 (0.59) **F = 6.24, p<0.02** No significant difference in errors of commission for "press for X" was found. All particpants were able to perform the task w/ >85% accuracy *Whole brain imaging analysis:* A ss negative assoc was seen btwn amount of prenatal Cnb exposure and neural activity during response inhibition in the anterior lobe of the left cerebellum after correcting for multiple comparisons at a threshold value of **p<0.001**	<u>Included:</u> Prenatal nicotine, alcohol and caffeine exposure Current Cnb, nicotine and alcohol use IQ	13 participants tested positive for Cnb in their urine; a one-way ANOVA showed no differences btwn exposed and unexposed groups on the basis of amount of Cnb in their urine. The authors noted that prenatal Cnb exposure is significantly related to altered bilateral prefrontal cortex activity corresponding to the amount of prenatal Cnb exposure. Authors postulate that since prefrontal cortex activity tends to increase when a task is difficult, the observed increase in prefrontal cortex activity might be a compensatory process in prenatally exposed participants where they recruit a wider functional neural network to perform the task accurately. A ss positive assoc was observed btwn amount of prenatal Cnb exposure and neural activity in the

Study/ Location	Study Design, Sample Sizes, Statistical Analysis	Outcomes of Interest	Exposure Measurement Methods	Exposure quantification	Results	Covariates/ Confounders	Comments
	ANCOVA, multiple regression				*Regions of Interest:* Regions w/ neural activity during response inhibition that were positively related to amount of prenatal Cnb exposure were located in the frontal/prefrontal cortex (included the right inferior frontal gyrus/premotor cortex and the left lateral orbital frontal gyrus) *Analysis controlling for current Cnb use:* As a control for the 13 participants w/ Cnb in their urine sample, a separate analysis was conducted on the 18 participants w/ a negative urinalysis Authors observed trends btwn amount of prenatal Cnb exposure and neural activity, in the same areas where differences were seen for the analyses that included all the 31 participants (regions of the left cerebellum, left lateral orbital frontal gyrus and right inferior frontal gyrus/premotor cortex). However, the sample size of 18 did not have enough statistical power to produce ss		right premotor cortex which is involved in "response inhibition, response competition and the preparatory process leading to correct initiation or suppression of movement". <u>Strengths:</u> Statistical power was calculated to ensure sufficient sample size. Controlled for current drug use, including tobacco and alcohol use, in the analyses, using results from urine tests and self-report. <u>Limitations:</u> Authors noted that the use of an event-related design rather than the block design, would have been better for determining if the increased premotor activity is due to increased response inhibition rather than increased response preparation. Authors controlled for current Cnb use by conducting a separate analysis on participants w/ negative urine analysis

Human Neurodevelopmental Studies: Visual Function and Processing

Study/ Location	*Study Design, Sample Sizes, Statistical Analysis*	*Outcomes of Interest*	*Exposure Measurement Methods*	*Exposure quantification*	*Results*	*Covariates/ Confounders*	*Comments*
							(n=18). However, the small sample size limited the statistical power.

Appendix Table 2.3 Human Neurodevelopmental Studies: Visual Function and Processing

Study/ Location	*Study Design, Sample Sizes, Statistical Analysis*	*Outcomes of Interest*	*Exposure Measurement Methods*	*Exposure quantification*	*Results*	*Covariates/ Confounders*	*Comments*
Chakraborty et al. (2015) Auckland, New Zealand	Prospective cohort 2006 – 2010 (Wouldes et al. 2014) N = 170 Independent or hospital based midwives referred mothers to the study Participants were recruited based on methamphetamine use. Control group included both non-drug exposed children and children exposed to drugs other than methamphetamine Methamphetamine-exposed and non-exposed children were matched on SES,	Global motion perception in 4.5 yos Measured with random dot kinematograms: children viewed dots moving within a circle and indicated the direction in which the dots were mostly moving Global motion perception is a behavioral measure of processing w/in the dorsal extrastriate visual cortex that is believed to be particularly vulnerable to abnormal development, and is assoc w/ deficits in visuomotor tasks involving reaching, grasping, and locomotor action Delayed motor development at birth and btwn 1 to 3 yrs of age previously observed in the cohort served as further	Drug use assessed using Substance Use Inventory interview Meconium analysis using GC-MS	Frequency categorized as: <1 day/wk 1-4 days/wk 5-7 days/wk Amount of Cnb (w/ shared joints taken into account) categorized as: Light: <1 joint/ occasion Moderate: 1-2 joints/ occasion Heavy: >2 joints/ occasion Single estimates of amount and frequency were obtained by ranking amount	Children exposed to Cnb had better motion coherence thresholds than children not exposed to Cnb (**t_{163}=3.52, p=0.001**) There was a ss interaction btwn effects of prenatal alcohol exposure and Cnb exposure on motion coherence thresholds ($F_{1,114}$=7.7, p=0.006). Exposure to Cnb in the absence of alcohol was ss assoc w/ improved global motion perception compared to children w/ no prenatal drug exposure (**t_{41}=4.42, p<0.001**) Exposure to Cnb and alcohol together was assoc w/ global motion perception that was no different than no drug exposure (t_{70}=-0.28, p=0.39)	<u>Included:</u> Other drug use (nicotine and methampheta-mine) Verbal IQ (based on WPPSI-III) Sex Ethnicity Habitual visual acuity Stereoacuity Alcohol as an effect modifier	Children exposed to alcohol prenatally had poorer global motion perception. However, Cnb co-exposure seemed to make this assoc ns. <u>Strengths:</u> Multiple exposure measurement methods <u>Limitations:</u> Majority of children were exposed to multiple drugs. Only 25 were in the non-drug comparison group

maternal education, BW, and GA n = 165 Univariate general linear model, multiple linear regression	motivation to investigate this outcome	and frequency for each trimester (e.g., 1, 2, or 3 for light, moderate, or heavy use, respectively) and taking the median of the ranks for all trimesters. Categories were dummy coded 81.3% of sample were exposed to multiple drugs 40% were exposed to Cnb (percent exposed to Cnb alone not specified) 15% exposed to no drugs	Multiple linear regression on children exposed to Cnb but not alcohol showed that frequency of maternal use (**β =-0.90; $F_{3,16}$=28.19, p <0.001; adjusted R^2=0.75**) and amount consumed per occasion (**β= -0.89; $F_{3,16}$=33.26, p<0.001; adjusted R^2=0.78**) each had a negative linear assoc w/ motion coherence threshold, indicating improved global motion perception

Study/ Location	***Study Design, Sample Sizes, Statistical Analysis***	***Outcomes of Interest***	***Exposure Measurement Methods***	***Exposure quantification***	***Results***	***Covariates/ Confounders***	***Comments***
Fried and Watkinson 2000 Ottawa, Canada OPPS	Prospective cohort N = 698 preg women <u>Eligibility Criteria:</u> Used any Cnb during preg Drank alcohol ≥ 0.85 oz/day during preg Smoked ≥16 mg nicotine/day during preg n = 140 resulting cohort of 9-12 yo Additional 50 children of women who did not use Cnb, drank little or no alcohol, and did not smoke n = 190 Attrition due to families moving out of Ottawa area, withdrawal, and children's unavailability for testing n = 146 9-12 yo 82 boys, 64 girls	Visuoperceptual performance in 9-12 yo children assessed through several tests: *Test of Visual-Perceptual Skills (TVPS):* a nonmotor task w/ a composite score (Perceptual Quotient) w/ 7 subscales: Visual Discrim-ination; Visual Memory; Visual Sequential Memory; Visual Form Constancy; Visual Sequential Memory; Visual Figure-Ground: Visual Closure These tests assess the ability to process and recall various characteristics of shapes using a variety of methods *Trail making test* *Knox Cube* *Wechsler Intelligence Scale for Children- III Perceptual Organization Index* w/ 4 subscales of problem solving (Block Design, Object Assembly, Picture	Maternal self-report Interviews conducted in woman's home during each trimester after enrolling in study Data were collected on quantity and pattern of drug use, caffeine use, age, height, pre-preg weight, weight gained during preg, regular maternal exposure to the cigarette smoke of others, general health, history of previous pregs, a 24-hr dietary recall, father's medical history, level of education, and family's SES	Maternal average Cnb use across preg categorized into three groups: No use: 0 joints Infrequent/mod-erate use: >0 and <6 joints/wk Heavy use: ≥ 6 joints/wk Cigarette use: a nicotine score was determined by multiplying number of cigarettes smoked by the nicotine content of the specific brand Maternal cigarette and Cnb use was dichotomized (cigarette smoking/non-smoking, and Cnb heavy/less	After adjusting for other maternal drug use, the strongest negative assoc found using linear trend analysis (t) were for: Perceptual Organization Index of WISC-III (**linear trend t = -2.2, p <0.05**) Object Assembly subscale (**linear trend t = -2.2, p<0.05**) After controlling for the Perceptual Quotient of TVPS, prenatal Cnb exposure remained negatively assoc w/: Perceptual Organization Index of WISC-III (**linear trend t = -2.3, p <0.05**) Block Design subscale (**linear trend t = -2.4, p <0.05**) Object Assembly subscale (**linear trend t = -2.0, p <0.05**) None of the TVPS subscales were assoc w/ prenatal Cnb exposure Assoc btwn prenatal Cnb exposure and overall Perceptual Quotient summary score was ns	Variables were assessed for assoc w/ Cnb use at p≤0.10. These variables were then examined for assoc w/ outcome variables and were included in the final model if p≤0.05 <u>Included:</u> Other maternal drug use <u>Considered:</u> Parental education Prenatal passive smoke exposure Sex of baby <u>Postnatal variables</u> <u>Included</u>: Postnatal passive smoke exposure <u>Considered:</u> Home environment Current SES Child's gender	Authors note that the subsets requiring skill in planning, integration, analysis, and synthesis (fundamental abilities to problem-solving) in addition to basic visuoperceptual abilities were negatively assoc w/ prenatal Cnb use and that these findings are consistent w/ impacts on aspects of executive functioning. The authors note these findings suggest that prenatal Cnb use is "negatively assoc w/ performance in situations which demand "top-down", integrative visuoperceptual processing—the type of neurocognitive requirement underlying executive function". A dose dependent negative assoc was seen btwn prenatal cigarette exposure and an overall score reflecting basic visuoperceptual functioning. <u>Strengths:</u> Sample size allowed for

Study/ Location	Study Design, Sample Sizes, Statistical Analysis	Outcomes of Interest	Exposure Measurement Methods	Exposure quantification	Results	Covariates/ Confounders	Comments
	No differential loss of subjects w/ respect to drug variables ANOVA, trend analysis, polynomial analysis for linear trends	Completion, Picture Arrangement) and subscales Symbol Search. Mazes, and Coding Additional tests to assess abilities that might influence performance on visual tasks: *WISC Full Scale*, (measure of general intelligence); *WISC Digit Span* (test of memory); *WISC Freedom of Distractibility* (measure of attention; *Developmental Test of Visual-Motor Integration*		than heavy use) so as to assess the interaction of the two using factorial ANOVA	Factorial ANOVAs w/ maternal Cnb and cigarette use as independent variables show no potentiating effect btwn the two Trend analysis showed a dose dependent negative assoc btwn prenatal cigarette exposure and an overall score reflecting basic visuoperceptual functioning The assoc remained after consideration of both pre- and postnatal secondhand smoke exposure, and the nonperceptual demands of the tasks	WISC Full Scale WISC Digit Span WISC Freedom from Distractibility Developmental Test of Visual Motor Integration	detection of a medium effect size w/ 90% power. Extensive assessment and control for pre and postnatal tobacco smoke exposure. The same interviewers were used throughout the pregnancy.

Study/ Location	*Study Design, Sample Sizes, Statistical Analysis*	*Outcomes of Interest*	*Exposure Measurement Methods*	*Exposure quantification*	*Results*	*Covariates/ Confounders*	*Comments*
O'Connell and Fried 1991 Ottawa, Canada OPPS	Prospective cohort N not stated Participants were recruited through notices in obstetricians offices and public media and interviewed about prescription and other drug use, health history, and diet All 6-9 yo children whose mothers consumed >1 Cnb joint/wk during preg were selected (n exposed = 28) Children in control group (no maternal use of Cnb in preg) were matched w/ exposed children based on maternal tobacco and alcohol use during preg (n unexposed = 28) n = 56 The same examiner conducted the child assessments and was blind to maternal drug history Discriminant function analysis, path analysis, multivariate analysis of	Neurobehavioral development in 6-9 yo offspring, incl. ratings of behavior problems, visual-perceptual tasks, language comprehension, and distractibility Test battery included: Global intellectual measures (WISC-R) Test of Visual Perceptual Skills Visual-motor integration (Test of Visual Motor Integration, Draw-A-Man, and Trail Making Test) Attention and memory tasks	Maternal self-report	Regular Cnb use (avg >1 joint/wk during preg) was compared with no Cnb use during preg Exposure ranged from 1.5 to 50 joints/wk among Cnb users	Discriminant function analysis: 7 outcome variables discriminated between the Cnb users and nonusers, explaining 36% of the group variance: Syntax Quotient Stroop Interference Visual Discrimination Visual Sequential Memory Trail Making Test-Part A Anxiety Conduct problems Effect of maternal Cnb use was evident in increased conduct problems (**F=7.39, p=0.01)** and to a lesser degree, poorer visual sequential memory (F=3.13, p=0.08) and poorer visual discrimination (F=2.89, p=0.09) After controlling for covariates, interaction of Cnb use and maternal age was assoc w/ outcomes (p=0.01), principally visual sequential memory (F(1,46)=8.69, p=0.01) and syntax quotient (F(1,46)=4.11, p=0.05): offspring of younger Cnb users scored lowest and children of younger control women scored highest on tests. The effect of Cnb use in	Included: Aggression-external Aggression in the home Supervision Agreeableness Conscientious-ness Neuroticism Mother's age at delivery Considered: Location of test HOME questionnaire variables Maternal and, paternal education Income Child's health School progress Family size Birth order Principal languages at home and school Mother's personality and intelligence Other sociodemo-graphic information	Authors note that univariate trends in this study are consistent with previous reports on young children, e.g., language comprehension scores, memory test scores, and ratings of child behavior were lower for children of Cnb users Strengths: Authors calculated 84% statistical power based on an effect size of 0.4 standard deviations Limitations: Sample size too small to permit extensive matching of subjects on potentially important confounders Authors note that the main findings were related to parental rating of child behavior problems. Whether these findings indicate true behavioral differences in children or different levels of parental tolerance is unclear. Postnatal Cnb exposure was not assessed in this study 6 women in the Cnb user group and none of the

Study/ Location	Study Design, Sample Sizes, Statistical Analysis	Outcomes of Interest	Exposure Measurement Methods	Exposure quantification	Results	Covariates/ Confounders	Comments
	covariance, multiple regression	(Stroop Color and Word Test, Knox Cube Test) Motoric assessment (Finger Tapping Test) Language comprehen-sion (Syntax Quotient derived from the Test of Language Development-Primary) Academic achievement (3 subtests of the Wide Range Achievement Test-Revised, Passage Comprehen_ sion subtest of the Woodcock Reading Mastery Test) Parent ratings of behavior problems			this model was diminished (p=0.11). When the 6 mothers who reported other illicit drug use were excluded from analysis, the main effect of Cnb use on discriminating outcome variables was ss **(p=0.02),** as was the interaction btwn Cnb use and maternal age at delivery **(p=0.05)** Path analysis: In analysis of conduct problems, of the 6 covariates, only aggression in the home was ss (Beta weight **B=0.51, p=0.02)**. Including aggression in the home with maternal Cnb use results in a ss model (**R^2=0.28, p=0.0002)**. The combined indirect and direct effect of maternal Cnb use on conduct problems was 0.43, which approximated the zero-order correlation btwn the two variables (r=.39). No dose-response relationship was detected		non-users reported use of other drugs in preg (all infrequent, mostly cocaine).

Study/ Location	***Study Design, Sample Sizes, Statistical Analysis***	***Outcomes of Interest***	***Exposure Measurement Methods***	***Exposure quantification***	***Results***	***Covariates/ Confounders***	***Comments***
Smith et al. (2006) Ottawa, Canada OPPS Same population as in Smith et al. (2004 and 2016)	Prospective cohort Preg women were recruited from 1979-1983 Participants were randomly selected from OPPS offspring <u>Eligibility criteria</u>: Completed the WAIS-III and C-DISC Clinical Test w/in 1 yr prior to the study, 18-22 yo, Right handed, No Axis I DSM-IV diagnosis, No structural abnormalities, English as the first language N = 35 4 participants were excluded due to current cocaine, opiates or amphetamine use (as per urine test, or self-reported use defined as ≥1x/mo)	Visuospatial working memory in 18-22 yo Tests included: Visuospatial n-back task (adapted from the standard n-back) assessed visuospatial working memory, Match to Centre task was the control condition Tests were performed while fMRI was conducted to assess brain activity during tests “Twelve regions were selected from brain areas previously reported to be involved in visuospatial working memory processing, specifically from previous n-back fMRI studies.” *explanation of tests included in the neurodevelopmental subsection of section D.**	Maternal self-report Prenatal exposure measurements were not specified in this article, therefore exposure measurement methods were retrieved from another OPPS article, Fried and O’Connell et al (1987): Interview during each trimester conducted in the mother’s home (as detailed in Fried et al. 1980; Fried and O’Connell et al. 1987)	Prenatal Cnb exposure defined as regular maternal use of at least 1 joint/wk for the entire preg n=15 non-exposed n=16 exposed (joints/wk): Range = 0.33-54 Mean (SE) = 8.27 (3.24) Exposure was analyzed as a continuous variable	*Whole brain analysis:* Negative ss assoc btwn amount of prenatal Cnb exposure and activity in right precentral gyrus (**z=6.8, p=0.038** This assoc remained ss after correcting for multiple comparisons at p = 0.00 Negative ss assoc btwn prenatal Cnb exposure and left putamen (**z=4.2, p=0.007**, was ss at a corrected value of p=0.05 Positive ss assoc btwn prenatal Cnb exposure and neural activity in right cuneus (**z=5.27, p=0.008**) *Neural activity in regions of interest during visuospatial working memory:* Positive ss assoc btwn prenatal Cnb exposure and left medial prefrontal cortex (PFC) (**z=5.78, p=0.015)** Positive ss assoc btwn prenatal Cnb exposure and the left inferior frontal gyrus (**z=3.24, p=0.042**) Positive ss assoc btwn prenatal Cnb exposure and	<u>Included:</u> Prenatal nicotine, alcohol and caffeine exposure Current Cnb, nicotine and alcohol use IQ	Authors note that the most robust effect was a ss reduction in activity in the right precentral gyrus/premotor cortex w/ increasing levels of prenatal Cnb exposure. This brain region reflects the encoding process in visuospatial short term memory. Authors postulate that differences in brain activity among prenatally exposed participants may be due to strategic differences in the approach to performing the task, or a compensatory response where left brain systems are increasingly engaged to compensate for functionally compromised right brain systems. <u>Strengths:</u> Statistical power was calculated to ensure sufficient sample size. Correlation btwn self-reported drug use and the urine test for Cnb = 0.97, and for smoking = 0.91 (p < 0.001).

Study/ Location	Study Design, Sample Sizes, Statistical Analysis	Outcomes of Interest	Exposure Measurement Methods	Exposure quantification	Results	Covariates/ Confounders	Comments
	n = 31 ANCOVA, Multiple regression analysis				left cerebellum (**z=5.13, p=0.037**) Reduced activity w/ increased prenatal Cnb exposure in specific areas of the right prefrontal cortex, including: Dorsolateral PFC (**z=3.07, p=0.042**) Medial PFC **(z=3.31, p=0.046**) Ventral PFC **(z=2.86, p=0.02)** Significant decreases in activity were observed in the left presupplementary motor cortex (**z=3.69, p=0.037**) and the right parahippocampal gyrus (**z=4.63, p=0.014**) "All results for the ROI [region of interest] analyses were corrected at 0.05 w/in a 10 mm sphere of the predetermined ROI." *Controlling for current Cnb use:* As a control for the 13 participants w/ Cnb in their urine sample, a separate analysis was conducted on the 18 participants w/ a negative urinalysis		Cognitive performance was examined while controlling for important covariates to ensure that "no other drug effects were responsible for the effects observed and that IQ did not play a role in the results". <u>Limitations:</u> Authors controlled for current Cnb use by conducting a separate analysis on participants w/ negative urine analysis (n=18). However, the small sample size limited the statistical power.

Study/ Location	Study Design, Sample Sizes, Statistical Analysis	Outcomes of Interest	Exposure Measurement Methods	Exposure quantification	Results	Covariates/ Confounders	Comments
					Authors observed ss or trends towards ss paralleling those in the urine positive participants Reduced activity w/ increased prenatal Cnb exposure was found in the right precentral gyrus (**z=3.33, p=0.025**), left putamen, and left medial PFC Positively related activity w/ increased prenatal Cnb exposure was seen in the right visual cortex and cerebellum *Cognitive performance:* Differences observed btwn prenatally exposed and non-exposed on reaction time, errors of commission or omission (while controlling for prenatal nicotine, alcohol, and caffeine exposure, current Cnb, alcohol and nicotine use, and IQ) were all ns		

Study/ Location	*Study Design, Sample Sizes, Statistical Analysis*	*Outcomes of Interest*	*Exposure Measurement Methods*	*Exposure quantification*	*Results*	*Covariates/ Confounders*	*Comments*
Willford et al (2010a) Pittsburgh, PA MHPCD	Prospective cohort Participants recruited from prenatal clinic of Magee Women's hospital, 1983-1985 N = 1360 Women ≥18 yrs were approached at 4th prenatal month visit. Women who drank ≥3 alcoholic drinks/wk and women who used ≥2 joints/mo, and random samples of women who did not use as much alcohol or Cnb were selected. 829 women were selected At delivery, 763 mothers of live singletons remained, and at 16 yrs, 589 adolescents remained in the cohort. Attrition was due mainly to loss to follow-up, refusals, and moving out of the area. 269 adolescents did not complete the	Processing speed, visual-motor coordination, and interhemispheric transfer at 16 yrs, as assessed by the computerized BCT On the BCT, subjects moved a cursor through straight-line target paths at different angles on a computer monitor, controlling horizontal movement w/ the left knob and vertical movement w/ the right knob. Target pathways consisted of angles that required both hands to respond equally (bimanual symmetrical), the left to move faster than the right (bimanual asymmetrical) and vice versa, and only one hand to move (unimanual). 2 trials were completed for each pathway angle, for a total of 16 trials. Visual motor coordination was calculated as the speed to complete bimanual angles relative to unimanual (baseline) speed. Interhemispheric transfer was calculated by dividing	Maternal self-report Women were interviewed at the 4th and 7th mo of preg, at delivery, and at 8 and 18 mo, and 3, 6, 10, 14, 16, and 22 yr of age of offspring Mothers were assessed for substance use, lifestyle, current environment, medical history, demographic status, depression, anxiety, hostility, intellectual ability Adolescents assessed for physical and psychosocial characteristics, and alcohol, tobacco, Cnb, and other illicit drug use	Quantity, frequency and pattern of mother's Cnb use assessed at end of each trimester Cnb use summarized as avg joints/day Cnb use levels (% in 1st, 2nd, 3rd trimester) No use (59, 77, 80) Light/moderate use, <1 joint/ day (28, 17, 15) Heavy use, ≥1 joint/day (13, 5, 5)	Assoc for trimester of prenatal Cnb exposure (standardized β) 1st trimester: slower <u>processing speed</u> for the 22.5º asymmetrical angle, left dominant on the bimanual coordination task (**β=0.09, p<0.05**) 3rd trimester: faster reaction times for 135º angle, indicating better performance on symmetrical movement measures of <u>visual motor coordination</u> (**β=0.01, p<0.01**) Slower reaction time for summary measure for leftward dominant movement (**β=0.15, p<0.05**) and left dominant 157.5º angle (**β= 0.12, p<0.05**), indicating worse <u>interhemispheric motor coordination</u> No ss interactions between Cnb and prenatal tobacco, alcohol, or cocaine exposure	<u>Included:</u> Variables that were ss at p<0.10 were selected for analyses but not specified <u>Considered:</u> *Maternal characteristics*: IQ Income Education Work status Age Marital status Depression Anxiety Hostility *Adolescent characteristics*: Sex Race Number of life events Age Handedness Depression Anxiety Current exposure to tobacco, Cnb, alcohol, other illicit drugs IQ *Prenatal*	Prenatal Cnb use was associated with deficits in processing speed and interhemispheric transfer of information, and better performance in visual motor coordination. Authors note that this was the first study to evaluate the effects of prenatal Cnb use on processing speed, visual motor coordination, and interhemispheric transfer using a bimanual coordination task, therefore there is no precedent to the effects of Cnb exposure on these cognitive functions. <u>Strengths:</u> Authors note that assessment of covariates allowed understanding of larger context for effects of prenatal Cnb exposure <u>Limitations</u>: Covariates included in the analyses were not reported

Study/ Location	Study Design, Sample Sizes, Statistical Analysis	Outcomes of Interest	Exposure Measurement Methods	Exposure quantification	Results	Covariates/ Confounders	Comments
	Bimanual Coordination Test (BCT) due mainly to time constraints, phone interview, equipment failure, unavailability, and illness n = 320 Multiple linear regression	the time for bimanual asymmetrical angles by average speed of the symmetrical angles				*exposure, by trimester*: Alcohol Tobacco	

Studies that were not as informative as those tabulated above are summarized below.

Fried (1980) and Fried (1982), from the OPPS prospective cohort study, examined the associations between in utero Cnb exposure and nervous system abnormalities in infants up to 1 year old. Pregnant women were interviewed for information about Cnb use in the year before pregnancy and each trimester, and were categorized as nonusers or irregular (≤1 joint/wk *or* exposure to secondhand Cnb smoke), moderate (2-5 joints/wk), or heavy (>5 joints/wk) users.

In Fried (1980), 89 singleton infants were assessed 60-80 hrs postpartum using the Brazelton Neonatal Assessment Scale (BNAS). Compared to infants of non- and irregular Cnb users in unadjusted analyses, infants of moderate and heavy Cnb users were:

- more likely to show alterations in visual response (chi-squared test, **p<0.01**): less likely to respond to light (chi-squared p<0.08) and habituate to light (statistics not reported)
- not different in tests of auditory responses (statistics not reported)
- less able to self-quiet (chi-squared **p< 0.02**)
- no different in being consoled by the examiner (statistics not reported)
- no different in responsiveness when held (statistics not reported)
- more apt to marked tremors (Mann-Whitney **p=0.008**)
- more apt to marked startles (Mann-Whitney **p=0.023**).

All infants of the seven Cnb users who continued to smoke >5 joints/wk during pregnancy scored ≥7 on the tremulousness scale of the BNAS, whereas 28/82 of the remaining sample scored as high (chi-squared **p<0.01**).

No differences were observed in tests of lateralization, muscular tone, hand-to-mouth behavior, general activity, alertness, or lability of states (data not reported).

Fried (1982) matched moderate to heavy Cnb users with nonusers who had comparable nicotine and alcohol habits, and adds data from assessments at 4, 9, and 30 days, and 1 year after birth. Visual stimuli responsiveness, tremors, startles, and habituation to light were similar to nonusers at 30 days. The previously reported reduced success at self-quieting was not confirmed. Findings of no differences in responsiveness when held, being consoled, laterality of handedness, general activity, and alertness were confirmed. Cnb-exposed infants were not more irritable.

Comparisons of 7 children of moderate and heavy users to children of matched non-users revealed no differences in mental, motor, or behavioral scales, and no differences in attitudes, interests, or temperament (data not reported).

Cri du chat was noted exclusively among children of Cnb users in both reports, but was not systematically recorded.

Faden and Graubard 2000 evaluated the association between maternal cannabis use with developmental outcomes at three years of age. The study used data from the National Maternal and Infant Health Survey (NMIHS) with information on N = 13, 417 total live births. The sample size analyzed was n = 8,285, as these were the participants for which there was 3 year follow up data. The developmental outcomes assessed were language, gross and fine motor function, and adaptive behavior assessed by the Denver Development Scale. The study also evaluated specific behaviors such as eating problems, length of play, activity level, difficulty of management, level of happiness, fearfulness, ability to get along with peers, tantrums, and eating nonfood. The mothers reported child's behavior via questionnaires mailed to mothers. Drug use was categorized as no use, less than once a month, once a month, two or three times a month, one to two times a week, and more than three times a week. Cumulative logit analysis revealed that prenatal cannabis exposure was significantly associated with fearfulness (β=0.097, RR=1.34, p=0.04, CL 1.02-1.76) and poorer gross motor development (β=0.074, RR=1.25, p=0.02, CL 1.01-1.08). Relative risk was computed for a change in three "drug uses" per week. Shorter length of play was also associated with cannabis use, although not statistically significant (β=0.45, RR=1.57, p=0.08, CL 0.86-2.60).

Appendix Table 2.4 Human Neurodevelopmental Studies: Intelligence/ Achievement

Study/ Location	***Study Design, Sample Sizes, Statistical Analysis***	***Outcomes of Interest***	***Exposure Measurement Methods***	***Exposure quantification***	***Results***	***Covariates/ Confounders***	***Comments***
Day et al. (1994b) Pittsburgh, PA MHPCD	Prospective cohort Participants recruited from prenatal clinic at Magee-Women's Hospital 1983 - 1985 N = 1,360 (refusal rate was 15%) 2 cohorts selected: Women who drank ≥3 alcoholic drinks/wk in the 1st trimester and a random sample of 1/3 of women who drank <3 drinks Women who used Cnb ≥2 times per mo in the 1st trimester, and a random sample of 1/3 of women who used <2 times/mo Both cohorts were combined for these analyses n = 829 (combined cohort) <u>Eligibility Criteria:</u> live singleton birth	Cognitive development of offspring at 3 yrs assessed by the Stanford-Binet Intelligence Scale (SBIS Composite, verbal reasoning, abstract/visual reasoning, quantitative reasoning, and short-term memory scores obtained from this test Assessment personnel were trained on reliability and were supervised by developmental psychologists and were blind to maternal substance use	Maternal self-report Mothers interviewed at 4th and 7th mo of preg and at delivery Offspring examined at delivery, 8 mo, 18 mo, 3 yrs and 6 yrs At each follow-up, mothers were interviewed w/ a standardized instrument that measured alcohol, Cnb, tobacco, and other illicit drug use. Additional information about demographics, lifestyle, environment, medical history, and psychological status of mother was also collected. Cognitive	Interview assessed usual, minimum and maximum quantity and frequency of Cnb, hashish, and sinsemilla Hashish use = 3 Cnb joints Sinsemilla use = 2 Cnb joints Cnb use expressed as average daily joints (ADJ) Cnb use was calculated for each mo of the 1st trimester, and was reported over the whole trimester for the 2nd and 3rd trimester Prevalence of mother's prenatal heavy Cnb use (≥1 ADJ) 1st trimester	Stepwise regression assessing assoc btwn prenatal Cnb exposure by trimester and SBIS (coefficient (β) = effect assoc w/ a change of 1 joint/day) (presented p values are one-tailed values) No significant assoc btwn composite Stanford-Binet score for any trimester or current maternal use When subscale scores were used as outcome variables, 2nd trimester (β = -1.5, p = 0.06) and current (β = -1.3, p = 0.06) Cnb exposure was marginally significantly assoc w/ short-term memory Interaction term btwn ADJ and race was ss; thus analyses were then run separately by race: African-American: 1st trimester: Verbal reasoning **-1.3 (p=0.007)** IQ points/joint/day 2nd trimester: Short-term memory: **-1.6 (p=0.05)** IQ points/joint/day White: no effects on composite or subscale scores Interaction term btwn ADJ and preschool/day care attendance	<u>Included:</u> Race Parity Marital status Maternal education Maternal depression, anxiety, and perception of child's difficultness Social support Child's school and day-care attendance <u>Considered:</u> Maternal age Sex of child Maternal work status Family income Maternal hostility Maternal self-esteem Household structure Number of siblings Distance in age btwn siblings Organization of household Stimulation in	This study found prenatal Cnb exposure was assoc w/ significantly decreased scores on the Stanford-Binet. The decrease was offset by preschool/day-care attendance for white children, but not for African-American children. Impact of prenatal Cnb exposure was greater than the effect of current Cnb use by mother. Majority reported alcohol and tobacco use. Sample was evenly divided by race. Women were generally of lower SES. Average composite score was 96 (range 72-131). <u>Strengths:</u> Prospective study design minimizes recall bias and establishes temporal sequence of events. Tested for interactions btwn Cnb and significant predictors of performance. Assessed influence of exposure at 3 yrs.

Study/ Location	Study Design, Sample Sizes, Statistical Analysis	Outcomes of Interest	Exposure Measurement Methods	Exposure quantification	Results	Covariates/ Confounders	Comments
	n = 763 (examined at birth) Attrition due to fetal death, refusal, loss to follow up, moving out of the region, adoption, or multiple births n = 672 subjects interviewed at 3 yrs Exclusions due to late assessment of child, child refusal to complete test, or physical impairments in child n = 655 (final cohort) Substance use patterns in mothers of participants did not differ from mothers of children who were not able to participate Regression model		development was assessed at 3 yrs	=14%, 2nd and 3rd trimester use = 4%	was ss; thus, an interaction term was entered into regression models: Total cohort: 1st trimester: Composite: **1.6** (interaction) Short-term memory: **3.0** (interaction), **-1.1** (main) Verbal reasoning: **1.9** (interaction) 2nd trimester: Short-term memory: **-2.3 (p=0.02)** (main) African-American 1st trimester: Composite: **-0.9 (p=0.05)** (main effect) Short-term memory: **-1.1 (p=0.05)** (main) Verbal reasoning: -**1.5 (p=0.005)** (main effect) 2nd trimester: Short-term memory: **-1.8 (p=0.05)** (main) White: 1st trimester: Short-term memory: **3.6** (interaction) 2nd trimester: Composite: **8.9** (interaction), **-8.9 (p=0.007)** (main) Verbal reasoning: 8.9 (interaction), -8.6 (main) Abstract/visual: **-7.6 (p=0.03)** (main)	household Life events Use of Cnb, alcohol, tobacco, and other illicit drugs at 3 yrs postpartum Trimester-specific use of Cnb, alcohol, tobacco, and other illicit drugs	<u>Limitations:</u> Authors note that factors in the current environment may be correlated w/ prenatal Cnb use and that these may be determining the findings. Not all results were reported.

Study/ Location	Study Design, Sample Sizes, Statistical Analysis	Outcomes of Interest	Exposure Measurement Methods	Exposure quantification	Results	Covariates/ Confounders	Comments
					3rd trimester: Short-term memory: 13.8 (interaction), -8.9 (main) Prenatal Cnb use was compared w/ mother's current use at 3 yrs postpartum (use: higher (ADJ≥0.5) and lower (ADJ<0.5)) Scores on the Stanford-Binet were adjusted for significant covariates identified from regression analyses Adjusted composite score among children who were currently exposed to a higher amount of Cnb but who had been exposed to the lower amount during the 2nd trimester of preg = 98 *(*n = 47). In contrast, children who were currently exposed to a lower amount but who had been exposed to the higher amount during the 2nd trimester had a composite score of 93 *(*n = 17). Composite and short-term memory scores for the total cohort, African-Americans and whites separately, and w/i racial groups, by preschool/ day-care attendance were assessed Authors concluded that "In each comparison, the impact of prenatal exposure was greater than the effect of current Cnb use, although it was not always significantly different because of small cell sizes"		

Study/ Location	*Study Design, Sample Sizes, Statistical Analysis*	*Outcomes of Interest*	*Exposure Measurement Methods*	*Exposure quantification*	*Results*	*Covariates/ Confounders*	*Comments*
Fried and Watkinson 1988 Ottawa, Canada OPPS	Prospective cohort Most births 1980 - 1983 N = ~700 pregs, including all women who: used Cnb, consumed >0.85 absolute alcohol (AA)/day, or smoked >15 mg nicotine/day during preg, plus 50 women who used no Cnb or nicotine, and little or no alcohol n = 217 children followed to 12 mos n = 153 at 24 mos. Of these, 124 were also tested at 12 mos. Reasons for lack of data at 12 mos include families moving away and unavailability due to sickness or vacation. Loss of funding was one reason for lower participation at 24 mos Tests were administered by 2 trained examiners in the children's homes. Observers agreed on ≥92% of items and at least one observer was unaware of the mother's drug use history Factor analysis, multiple linear regression	HC Height Weight At 12 mos: Bayley Scales of Infant Development (BSID), including Mental Development Index (MDI;), Psychomotor Development Index (PDI), Infant Behavior Record (IBR) At 24 mos: BSID, Reynell Developmental Language Scales, HOME test	Maternal self-report Repeated interviews asking about drug use in the previous trimester of preg Of 124 children followed to 12 and 24 mos, 54 and 53, respectfully, were prenatally exposed to Cnb Prenatal Cnb use among children of users, mean (range): Assessed at 12 mos: 15 (0.3-153) joints/wk Assessed at 24 mos: 18 (0.3-153) joints/wk	Joints/wk (regression analyses) Categories (n for children followed to 12 and 24 mos, respectively): No use (162, 100) Heavy >5 joints/wk (17, 18)	HC: not reported for Cnb Prenatal Cnb use was assoc w/ increased child weight at 12 mos (**partial r=0.15, p<0.05**) and height at 24 mos (partial **r=0.17, p<0.05**) When Cnb use was categorized, mean growth measurements at 12 and 24 mos did not differ significantly for children of heavy Cnb users vs nonusers Cnb use was assoc w/ higher 12 mo IBR Primary Cognitive scores **(r=0.17, p<0.05)** Cnb use was assoc w/ lower 24 mo Reynell Comprehension Scores: **Pearson's r=–0.17, p<0.05** 12 and 24 mo MDI and PDI: No reported assoc w/ Cnb use No assoc btwn maternal Cnb use and 12 and 24 mo auditory or visual clusters	Considered (maternal unless otherwise stated): Caffeine, protein, caloric intake [n g] Alcohol use* [n] Nicotine use* [n] Preg difficulties (e.g., placenta previa) [n] Family income [n g] Age [n g] Education* [n g] Pre-preg weight [n g] Maternal and paternal health history [n] Parity* [n g] Exposure to Xrays, rubella [n] Infant BW [n g] GA* [n g] Breast vs. bottle feeding [n g] HOME test* [n] Paternal, maternal height [g] * Included in Reynell Comprehension Scores analysis [n] neurobehavioral analyses [g] growth analyses	Cnb use was negatively assoc w/ the HOME Test (r=-0.22, p<0.01) and 5 of 6 of its subscales: Emotional/verbal responsivity to mother Organization of the Physical and Temporal Environment Provision of Appropriate Play Materials Maternal Involvement w/ Child Opportunity for Variety in Daily Stimulation Limitations: Adjusting for HOME test, GA, and BW may be inappropriate if these variables are causally assoc w/ both Cnb use and neurobehavioral outcomes.

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Fried and Watkinson 1990 Ottawa, Canada OPPS	Prospective cohort N = 698 Women recruited via signs in obstetricians' offices and public media. Most births occurred btwn 1980-83 <u>Eligibility Criteria:</u> Children of women who reported any use of Cnb during preg Children of any women who drank alcohol >0.85 oz average/day Children whose mothers smoked an average of ≥15 mg nicotine/day during preg Children of 50 women who were nonusers of Cnb, abstained or drank little alcohol, were nonsmokers n = 190 Exclusion from analysis due to families moving out of the Ottawa area, sickness,	Neurobehavior at 36 mos and 48 mos McCarthy Scales of Children's Abilities including the verbal, perceptual, quantitative, and general cognitive, memory and motor scales Reynell Developmental Language Scales At 48 mos Same as above in addition to: Tactile Form Recognition Task in which the child recognizing the shape of an object out of sight by feeling it Pegboard test in which the speed and accuracy of eye-hand coordination is measured. The test involves the child placing keyhole-shaped metal pegs into rows of matching holds in a board as quickly as possible. Speed and accuracy are assessed The Peabody Picture Vocabulary Test Form L measured receptive vocabulary	Maternal self-report by interview	Cnb use categorized into 3 groups: No or infrequent use (control group): <1 joint/wk Moderate use: >1 joint/wk and <6 joints/wk Heavy use: ≥ 6 joints/wk ANCOVA was used for examining effects of multiple drug use (Cnb, tobacco and alcohol) categorizing: Cnb as heavy (≥6 joints/wk) or no or lighter use	<u>36 mos analysis:</u> The motor and quantitative subscales of McCarthy produced 2 ss discriminant functions: 1st function: chi-square (4; n=133) = **13.6, p<0.01**; Wilks' lambda =0.90; 2nd function = chi-square (1; n=133) = **4.6, p<0.05** and a correct classification of 76.7%) accounting for 67% and 33% of the between-group variability 1) McCarthy motor subscale was responsible for the discriminant power of the 1st function (maximally separated the moderate use group with higher scores from both control and heavy Cnb use groups 2) McCarthy quantitative subscale was responsible for the discriminant power of the 2nd function (maximally separated heavy Cnb use group with lower scores from the control and moderate use groups MANCOVA After controlling for covariates, Cnb use remained ss assoc w/ motor scores (F (4,250)= **2.9, p<0.05**) but not w/ quantitative scores, providing discriminatory power (**F(2,125)=4.1, p<0.05**) After controlling for parity the Cnb use and outcome assoc remained ss (**F=4.0, p<0.01**). Two discriminant functions were ss, again- motor scores were higher for the moderate Cnb use group compared w/	<u>Included:</u> Family income Mother's age Mother's education Pre-preg weight Pregnancy weight gain Parity Home environment <u>Considered:</u> Mother's weight Nutrition Alcohol and tobacco use Offspring sex Gestation BW	The assoc btwn prenatal Cnb exposure and lower scores on verbal and memory subscales at 48 mos remained ss after controlling for Home environment. No potentiating effects of Cnb w/ nicotine or alcohol use were observed at either 36 or 48 mos analysis. <u>Strengths:</u> Trained examiners were unaware of the mother's drug use. <u>Limitations:</u> Authors note that the low risk sample may not be representative of other SES populations.

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	recent emotional trauma, physical handicap, lack of cooperation, severe cognitive disability n = 130 at 36 mos n = 123 at 48 mos No differential loss of subject w/ respect to drug variables occurred No assoc noted btwn the drug habits of the mothers and reasons for invalid test Multivariate analyses, Discriminant function analysis				the other two, and quantitative scores were lower in the heavy Cnb use group compared to the other two After controlling for the home environment Cnb use remained ss assoc w/ outcome measures (**F=2.7, p<0.05**) and motor scores remained ss **w/ F=4.0, p<0.05.** Quantitative scores did not contribute to the discriminatory power in this model <u>48 mo analysis:</u> The Peabody Vocabulary test and the McCarthy memory and verbal subscales combined to provide two ss discriminant functions: **combined chi-square=17.8, p<0.01**, **2nd function chi-square=6.8, p<0.05**, w/ a correct classification of 76.1%): 1st function showed heavy Cnb use group w/ ss lower mean scores from non and moderate use group 2nd function showed ss lower memory scores on the McCarthy subscale for the moderate group MANCOVA After controlling for home environment Cnb use remained ss assoc w/ outcome variables F=2.3, p<0.05. The heavy use group had poorer memory skills than the moderate and non use groups		

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Fried et al (1997) Ottawa, Canada OPPS	Prospective cohort Pregnant women (n=690) volunteers were recruited through media and notices in obstetricians' offices. N=190 offspring selected for follow-up after birth. <u>Eligibility Criteria:</u> Children were 9-12 years old. 140 children whose mothers used any Cnb during preg, drank alcohol ≥ 0.85 oz/day during preg, and smoked ≥16 mg, nicotine/day during preg 50 children whose mothers did not use Cnb, drank little or no alcohol, and did not smoke English at home and at school, no Ritalin Attrition due to families moving out of Ottawa area, withdrawal, and children's unavailability for testing n = 131 children available for analysis (number of exposed vs unexposed was not given) No differential loss of subjects with respect to drug variables Multivariate analyses, stepwise discriminant function analysis	Reading and language assessed by: WISC-III and the Information, Similarities, and Vocabulary, and Verbal subscales, Full Scale IQ, and the Verbal Comprehension Factor score Wide Range Achievement Test-Revised reading subtest to assess single word reading recognition Peabody Picture Vocabulary Test to assess auditory comprehension of picture names Fluency Test to measure oral fluency Passage Comprehension subtest from Woodcock Reading Mastery Test requires the subject to read a short passage and supply key words Oral Cloze task to measure English auditory processing Seashore Rhythm test to assess the ability to discriminate between different patterns of nonverbal sounds Regular and Exception Pseudoword task to assess aspects of reading proficiency Psychological questionnaires to determine child's level of depression and anxiety	Maternal self-report Interviews conducted in home of participant during each trimester remaining in pregnancy after entrance into study Data collected on patterns of maternal drug use, maternal caffeine use, maternal age, height, pre preg weight, weight gained during preg, maternal exposure to secondary smoke, general health, history of previous preg, diet, and father's demographics and medical history	Cnb use recorded as number of joints/wk Group 1: ≤1 joint/wk Infrequent/ moderate use: >1 and <6 joints/wk Heavy use: ≥ 6 joints/wk	Maternal Cnb use was not associated with deficits in reading or language in 9-12 year old children Analysis with ANOVA did not show any significant assoc between reading outcomes and Cnb exposure groups Stepwise Discriminant Function analysis (DFA) on the six reading variables and the 3 Cnb exposure groups resulted in one significant discriminant function (chi-square = 13.2, $p<0.05$, Wilk's lambda – 0.90, 76% correct classification) accounting for 83% of total discriminatory variance The main discriminating variables were total correct and phonological scores from the Pseudoword task which separated poorer performance by moderate Cnb group from the control and the heavy Cnb group. The moderate Cnb group performed better on the orthographic task than the heavy and control Cnb groups After adjusting for pregnancy weight gain, maternal cigarette smoking, and alcohol use, the relationship between all Cnb groups and reading discriminant scores remained significant F = 3.6, $p>0.05$ Analysis with ANOVA did not show any significant assoc between language outcomes and Cnb exposure groups DFA analysis also was ns for the eight language variables	<u>Included:</u> Alcohol and cigarette use <u>Considered:</u> Family income Maternal age at delivery Maternal pre preg weight Average level of parental education Other maternal drug use Prenatal passive smoke exposure Sex of offspring Home environment Mother's personality Offspring depression and anxiety Secondhand smoke exposure of child Current maternal socio-demographic characteristics Maternal Cnb use at time of child's testing	The authors note they could not explain the unexpected result for offspring with moderate Cnb exposure performing more poorly on aspects of the pseudoword task as compared with the control or heavy Cnb users may be a chance finding. <u>Strengths:</u> The sample size was sufficient to obtain 90% statistical power in analysis. A single interviewer was used, minimizing interviewer bias. Testers were blinded to child's exposure status, minimizing differential information bias.

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Fried and Watkinson 2000 Ottawa, Canada OPPS	Prospective cohort N = 698 preg women <u>Eligibility Criteria:</u> Used any Cnb during preg Drank alcohol ≥ 0.85 oz/day during preg Smoked ≥16 mg nicotine/day during preg n = 140 resulting cohort of 9-12 yo Additional 50 children of women who did not use Cnb, drank little or no alcohol, and did not smoke n = 190 Attrition due to families moving out of Ottawa area, withdrawal, and children's unavailability for testing n = 146 9-12 yos 82 boys, 64 girls No differential loss of subjects w/ respect to drug variables ANOVA, trend analysis, polynomial analysis for linear trends	Visuoperceptual performance in 9-12 yo children assessed through several tests: *Test of Visual-Perceptual Skills (TVPS):* a nonmotor task w/ a composite score (Perceptual Quotient) w/ 7 subscales: Visual Discrim-ination; Visual Memory; Visual Sequential Memory; Visual Form Constancy; Visual Sequential Memory; Visual Figure-Ground: Visual Closure These tests assess the ability to process and recall various characteristics of shapes using a variety of methods *Trail making test* *Knox Cube* *Wechsler Intelligence Scale for Children- III Perceptual Organization Index* w/ 4 subscales of problem solving (Block Design, Object Assembly, Picture Completion, Picture Arrangement) and	Maternal self-report Interviews conducted in woman's home during each trimester after enrolling in study Data were collected on quantity and pattern of drug use, caffeine use, age, height, pre-preg weight, weight gained during preg, regular maternal exposure to the cigarette smoke of others, general health, history of previous pregs, a 24-hr dietary recall, father's medical history, level of education, and family's SES	Maternal average Cnb use across preg categorized into three groups: No use: 0 joints Infrequent/moderate use: >0 and <6 joints/wk Heavy use: ≥ 6 joints/wk Cigarette use: a nicotine score was determined by multiplying number of cigarettes smoked by the nicotine content of the specific brand Maternal cigarette and Cnb use was dichotomized (cigarette smoking/non-smoking, and Cnb heavy/less than heavy use) so as to assess the interaction of	After adjusting for other maternal drug use, the strongest negative assoc found using linear trend analysis (t) were for: Perceptual Organization Index of WISC-III (**linear trend t = -2.2, p <0.05**) Object Assembly subscale (**linear trend t = -2.2, p<0.05**) After controlling for the Perceptual Quotient of TVPS, prenatal Cnb exposure remained negatively assoc w/: Perceptual Organization Index of WISC-III (**linear trend t = -2.3, p <0.05**) Block Design subscale (**linear trend t = -2.4, p <0.05**) Object Assembly subscale (**linear trend t = -2.0, p <0.05**) None of the TVPS subscales were assoc w/ prenatal Cnb exposure Assoc btwn prenatal Cnb exposure and overall Perceptual Quotient summary score was ns Factorial ANOVAs w/ maternal Cnb and cigarette use as independent variables	Variables were assessed for assoc w/ Cnb use at p≤0.10. These variables were then examined for assoc w/ outcome variables and were included in the final model if p≤0.05 <u>Included:</u> Other maternal drug use <u>Considered:</u> Parental education Prenatal passive smoke exposure Sex of baby <u>Postnatal variables</u> <u>Included</u>: Postnatal passive smoke exposure <u>Considered:</u> Home environment Current SES Child's gender WISC Full Scale WISC Digit Span	Authors note that the subsets requiring skill in planning, integration, analysis, and synthesis (fundamental abilities to problem-solving) in addition to basic visuoperceptual abilities were negatively assoc w/ prenatal Cnb use and that these findings are consistent w/ impacts on aspects of executive functioning. The authors note these findings suggest that prenatal Cnb use is "negatively assoc w/ performance in situations which demand "top-down", integrative visuoperceptual processing—the type of neurocognitive requirement underlying executive function". A dose dependent negative assoc was seen btwn prenatal cigarette exposure and an overall score reflecting basic visuoperceptual functioning. <u>Strengths:</u> Sample size allowed for detection of a medium effect size w/ 90% power.

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		subscales Symbol Search. Mazes, and Coding Additional tests to assess abilities that might influence performance on visual tasks: *WISC Full Scale*, (measure of general intelligence); *WISC Digit Span* (test of memory); *WISC Freedom of Distractibility* (measure of attention; *Developmental Test of Visual-Motor Integration* (measure of visuomotor coordination)		the two using factorial ANOVA	show no potentiating effect btwn the two Trend analysis showed a dose dependent negative assoc btwn prenatal cigarette exposure and an overall score reflecting basic visuoperceptual functioning The assoc remained after consideration of both pre- and postnatal secondhand smoke exposure, and the nonperceptual demands of the tasks	WISC Freedom from Distractibility Developmental Test of Visual Motor Integration	Extensive assessment and control for pre and postnatal tobacco smoke exposure. The same interviewers were used throughout the pregnancy.

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Fried et al. (1992b) Ottawa, Canada OPPS	Prospective cohort Most births 1980 - 1983 Preg women volunteered learning about the study from obstetrician or notices in public media Selection criteria for follow-up beyond birth: Children of women who reported: Any Cnb during preg, Drinking avg >0.85 oz absolute alcohol/day, Smoking avg ≥15 mg nicotine/day during preg (n=140) Children of 50 women who were nonusers of Cnb, nonsmokers, and drank little or no alcohol n = 135 included for analysis at 5 yrs n = 137 included at 6 yrs Sample attrition due mainly to families moving out of area or withdrawal from study No differential loss of subjects w/ respect to drug variables Discriminant function analysis	Cognitive and receptive language development at 5 and 6 yrs, assessed using: McCarthy Scales of Children's Abilities, including subscales: verbal perceptual quantitative general cognitive index memory motor Peabody Picture Vocabulary Test-Form L (a test of receptive vocabulary) Assessments were conducted by a trained examiner who was unaware of maternal drug use	Maternal self-report Interviews conducted in woman's home during each trimester after enrolling in study Same interviewer was used throughout preg Data were collected on: quantity and pattern of drug use, including Cnb, alcohol, cigarette, and other drug use; caffeine use; age; height; pre-preg weight; weight gained during preg; regular maternal exposure to the cigarette smoke of others; general health; history of previous pregs; a 24-hr dietary recall; father's medical history; level of education; and family's SES	Cnb described in number of joints/wk Cnb use categories: - Infrequent/no use; - Moderate use (>1 and <6 joints/wk); - Heavy use (≥6 joints/wk)	Maternal Cnb use was not associated with cognitive function in offspring at 5 and 6 yrs Discriminant function analysis indicated no combination of the McCarthy Scales and Peabody Picture Vocabulary test differed based on categories of Cnb use at either 5 or 6 yrs No potentiating effects for heavy use categories of any pair of drugs	Cnb was not included in multivariable analyses due to lack of discrimination among Cnb use categories by any outcome variables	Strengths: The same female interviewers were used throughout the pregnancy. Limitations: Authors note that this is a low-risk sample and that there is evidence to suggest that Cnb's effect is potentiated in higher risk environments Authors also state that the potency of Cnb preparations have increased several fold, which warrants caution when extrapolating data from this study to current Cnb use

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Fried et al. (1998) Ottawa, Canada OPPS	Prospective cohort Most births 1980 - 1983 N = 698 Pregnant women were recruited through obstetricians' offices and public media and interviewed Follow-up cohort initially included 140 children of women who: used Cnb, consumed >0.85 absolute alcohol/ day, or smoked ≥16 mg nicotine/day during preg, and 50 children of women who used no Cnb or nicotine, and little or no alcohol n=131 at 9-12 yo Exclusions and losses: family moved away, unavailable for testing, withdrew from study, French language, child taking Ritalin Age (n): 9 (3), 10 (70), 11 (34), 12 (24) Children were administered a large battery of tests, from which measures of cognitive and executive functioning were selected for the current analyses. Assessments were	Cognitive and executive function in 9-12 yo children, measured by: Wechsler Intelligence Scale for Children-III (WISC-III) w/ its subscales, composite, and derived scores Gordon Diagnostic Delay and Vigilance Tasks Category Test Auditory Working Memory Test Fluency Test Tactual Performance Test (total time to perform) Developmental Test of Visual-Motor Integration (reported only in Discussion)	Interviews during each remaining trimester of preg, asking about maternal Cnb, nicotine, caffeine, and alcohol use; nutrition; sociodemographic characteristics; health Subsequent interviews assessed postnatal exposure to Cnb smoke and other potential covariates	# joints/wk Categories: Heavy use (≥6 joints/wk) Infrequent/ moderate (>0 and <6 joints/wk) Nonuser Mean Cnb use for infrequent/ moderate and heavy users was 1.9 joints/wk and 16.1 joints/wk, respectively (used in trend analyses)	<u>Discriminant Function Analyses</u> Prenatal Cnb use assoc w/ aspects of <u>executive function</u> Main discriminating measures on which Cnb-exposed children performed more poorly than unexposed (structure coefficients): Category Test (0.49) Gordon Delay total responses (0.34) WISC-Block Design (-0.30) WISC-Picture Completion (-0.30) Gordon Delay efficiency ratio (-0.29) <u>Trend Analyses</u> Mean (SE) scores by prenatal Cnb use, adjusted for pre- and postnatal confounders: WISC Comprehension nonuser 10.7 (0.35) moderate 12.6 (0.78) heavy 11.9 (0.90) **p≤0.05 for quadratic trend** WISC Block Design nonuser 12.3 (0.36) moderate 12.3 (0.86) heavy 10.5 (0.96) p≤0.10 for linear trend WISC Object Assembly nonuser 11.2 (0.29) moderate 12.7 (0.67) heavy 10.4 (0.72) **p≤0.05 for quadratic trend**	<u>Included in DFA</u> Maternal age at delivery Prenatal nicotine use Prenatal alcohol use Current maternal Cnb use Variables included in trend analyses not specified <u>Considered</u> *<u>Prenatal</u>* Family status Family income Parent education Prenatal passive smoke exposure *<u>Postnatal</u>* Child's sex Home Environment Questionnaire (10 scales) Mother's personality (NEO 5 Factor Inventory) Family income Maternal education	No differential loss of subjects with respect to drug use variables Additional questionnaires used to examine children's anxiety and depression Authors' discussion: WISC-Block Design subtest requires perceptual organization, spatial visualization, and abstract conceptualization. WISC-Picture Completion subtest assesses ability to differentiate essential from nonessential details. Both potentially involve visuo-spatial and visuo-motor abilities, and higher-order cognitive processes, such as planning, impulse control, visuo-construction and visuo-analysis. To test basic spatial and visual motor functioning, the Developmental Test of Visual-Motor Integration was administered. The test was not assoc with prenatal Cnb exposure, and inclusion as a covariate did not diminish assoc btwn Cnb use and discriminant outcome scores, suggesting maternal Cnb use does not affect basic visual and motor functioning, but

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	administered without knowledge of prenatal drug exposure ANCOVA, Discriminant function analysis (DFA)				Gordon Vigilance (number correct) nonuser 91.9 (2.3) moderate 98.4 (5.3) heavy 107.8 (5.6) **p≤0.05 for linear trend** Gordon Vigilance Commissions (errors) nonuser 116.5 (2.7) moderate 112.3 (6.3) heavy 98.5 (6.8) **p≤0.05 for linear trend** Category Test Total Errors nonuser 86.5 (2.0) moderate 90.7 (4.4) heavy 96.4 (5.1) p≤0.10 for linear trend No assoc with Full Scale IQ or composite Verbal IQ		rather “higher order” cognitive processes. Authors also note that WISC-Block design and Picture Completion require visual analysis, hypothesis testing, and inhibition of prepotent responses, which is consistent with the Category Test, Gordon Delay Task, and Gordon Efficiency Ratio (correct responses/total responses). <u>Strengths</u> Discriminant function analysis was cross-validated

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Fried et al (2003) Ottawa, Canada OPPS	Prospective cohort N = 698 <u>Eligibility Criteria:</u> Children of women who reported any use of Cnb during preg Children of women who drank alcohol >0.85 oz average/day Children whose mothers smoked an average of ≥16 mg nicotine/day during preg Children of 50 women who were nonusers of Cnb, abstained or drank little alcohol, were nonsmokers English as primary language No Ritalin medication n = 190 Participants excluded due to moving from the Ottawa area, withdrawal, unavailability for testing n = 145 ANOVA, ANCOVA, Stepwise discriminant function analysis	Cognitive functioning in 13-16 yo offspring measured by: Wide Range Achievement test and Peabody Spelling assessed general reading and language skills Missing Numbers, Abstract Designs, Sentence Memory, and Knox Cube tasks assessed different aspects of auditory and visual memory Wisconsin Card Sorting Test and Stroop tests assessed inhibition of prepotent responding WISC-III assessed overall intelligence	Maternal self-report by interview Collected relevant exposures, demographic information, and father's information during interviews at mother-to-be's home Interviews were conducted by the same interviewer to maintain consistency and develop rapport	Maternal average Cnb use categorized into 2 groups: No use or infrequent/ moderate use: <6 joints/wk Heavy use: ≥6 joints/wk Tobacco use categorized into 3 groups: nonsmoking; light; heavy (≥16 mg nicotine/day, ~ 1 package of average strength) Postnatal secondhand tobacco smoke exposure = proportion of life in which the subject was regularly exposed at least 2 hrs/day both inside and outside the home	The discriminant function analysis resulted in a univariate structure w/ 1 variable- Abstract Designs (latency) A ss assoc was found btwn Abstract Designs latency and Cnb groups where offspring of heavy Cnb use groups displayed slower response times. This remained ss after controlling for covariates: adjusted mean (SE) None/light Cnb: 49.2 (0.8) Heavy Cnb**: 53.7 (1.8), p≤0.05** Performance on Peabody Spelling was also negatively assoc w/ maternal Cnb use after controlling for relevant factors: Adjusted mean (S.E.) None/light Cnb: 102.3 (1.0) Heavy Cnb**: 97.3 (2.2), p≤0.05**	<u>Included:</u> Maternal preg alcohol and drug use <u>Considered:</u> SES at time of birth Maternal age Maternal drug use Mother's secondhand tobacco smoke exposure during preg <u>Postnatal variables:</u> Current SES Current marital status Current maternal tobacco and Cnb use Home environment Secondhand tobacco smoke exposure of offspring Offspring sex Offspring tobacco smoking habits	No potentiating or ameliorative effects noted btwn maternal tobacco use and Cnb use on cognitive variables examined. <u>Strengths:</u> Controlled for maternal tobacco smoking during pregnancy as well as maternal secondhand tobacco smoke during pregnancy. Additionally, controlled for the offsprings' lifetime exposure to secondhand tobacco smoke and own tobacco use using self-report and urine analysis. Long-term follow-up of cohort. Active tobacco smoking by subjects was determined by self-report and urinalysis. Prenatal exposure to maternal nicotine use did not differ btwn the non/light and heavy Cnb exposed offspring. <u>Limitations:</u> Low risk sample may not be representative of other SES populations.

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Gold-schmidt et al. (2004) Pittsburgh, PA MHPCD	Prospective cohort Participants recruited from prenatal clinic at Magee-Womens Hospital 1982 - 1985 <u>Eligibility Criteria:</u> English speaking ≥ 18 yo 4th mo of preg N = 763 2 cohorts selected and combined for these analyses: All women who drank ≥3 alcoholic drinks/wk in the 1st trimester and the next woman interviewed who drank <3 drinks/wk All women who used ≥2 Cnb joints/mo and the next woman who used <2 joints/mo Women could be in either or both cohorts n = 606 Attrition to 606 women from original sample size due mainly to: loss to follow up, loss of custody, refusals, family moved out of	Academic achievement and underachievement at 10 yrs Child depression and anxiety as mediators of the assoc btwn academic achievement and prenatal drug exposure Interaction btwn prenatal Cnb and alcohol use and effect on academic performance Underachievement defined as a large disparity btwn intelligence and school achievement Academic achievement assessed w/ WRAT-R, PIAT-R reading comprehension subtest, and teacher's report. WRAT-R is a reading comprehension subtest of the Peabody Individual Achievement Test-Revised (PIAT-R) that provides a screening measure of reading, spelling, and arithmetic skills. Teachers were asked to rank student performance in language arts, history, math, and science, and academic attainment in general on a scale of 1 to 5 (1 - bottom 10%, 3 - average, 5 - top 10%)	Maternal interview assessing use for each mo of 1st trimester and across entire trimester for 2nd and 3rd trimesters, conducted at 4th and 7th prenatal months, 24-48 hours after delivery, and at 10-yr follow-up	Prenatal Cnb use calculated based on quantity and frequency of Cnb use for each mo of the 1st trimester and across the 2nd and 3rd trimesters To standardize the ∆-9-THC content of various forms of Cnb, a bowl or joint of sinsemilla was counted as 2 joints, and hashish as 3 joints Cnb exposure quantified as average daily joints Categories of Cnb use: Abstainers Light/moderate users (<1 joint/day) Heavy users (≥1 joint/day)	<u>Regression results</u> 1st trimester Cnb use dichotomized to heavy vs no use, and was assoc w/ poorer outcomes: <u>WRAT – R reading</u>: **β=-3, p<0.05** <u>WRAT- R spelling</u>: **β=-3.5, p<0.05** <u>Teachers' rating</u>: **β=-0.25, p<0.05** 1st trimester heavy Cnb use assoc w/ child's self-reported <u>depression</u> **(p=0.002)** and <u>anxiety</u> **(p=0.03)** symptoms. When child's depression and anxiety symptoms were included in analyses, 1st trimester heavy Cnb use was no longer assoc w/ academic performance 2nd trimester Cnb use was associated with lower <u>PIAT-R reading comprehension</u>: **β=-2.9, p<0.05** 2nd trimester heavy Cnb use was associated with lower <u>teacher evaluation</u> of performance: **β=-0.4, p<0.05** 2nd trimester Cnb exposure was associated with <u>underachievement</u>: **OR=2.0, CI (1.05, 3.8), p = 0.04** 3rd trimester Cnb exposure was not associated with any	<u>Included or considered:</u> Maternal age Education Family income Presence of adult male in household Ethnicity Working status Home environment Mother's custody of child No. of siblings Age spread btwn oldest and youngest child Child gender Child depression Child anxiety Maternal depression Maternal hostility No. of life events Support from friends Support from relatives Other prenatal substance use	Authors state 'the effects of 1st trimester Cnb use on achievement were explained entirely by the effects of prenatal Cnb use on the child's depression and anxiety.' The study population was of low SES and equally African American and White. No ss differences in prenatal substance use or demographic characteristics btwn women who were included in analysis and those who were not included <u>Strengths:</u> Authors state that large sample size permitted statistical control for a large number of factors. Authors note that substance use was measured in each trimester of preg, reducing memory and reporting bias. <u>Limitations:</u> Authors note that some potential covariates may not have been adequately controlled for such as motivation, social skills,

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	state, physical or mental disability or psychiatric problems w/o medication. 110 teachers did not return questionnaire about child's academic performance. 496 children had teacher assessments Mean age of children was 10.5 yrs, 44% were in 5th grade Multiple regression, logistic regression, structural equation modeling	Expected level of achievement measured by Stanford-Binet Intelligence Scale 4th edition 63 children were identified as underachievers in reading, spelling, or arithmetic			measures of academic achievement Cnb by alcohol interaction was NS for any academic achievement outcomes Structural equation modeling results: The WRAT-R and PIAT-R scales were combined into a standardized achievement score. 1st trimester Cnb exposure was assoc w/ the achievement score **(coefficient =-0.08, p <.05)** and teachers' rating **(coefficient =-0.07, p <.05)**. When child's psychological status was added to the model, it mediated the assoc btwn 1st trimester Cnb use and achievement: 1st trimester Cnb exposure → child's psychological status **(coefficient =0.17, p <.05)** → achievement scores **(coefficient =-0.23, p < 0.05)** and 1st trimester Cnb exposure → achievement scores was no longer ss (coefficient not reported) Structural equation modeling showed that 2nd trimester Cnb exposure was not assoc w/ achievement score and teacher's rating		and parent involvement in child's education.

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Gold-schmidt et al. (2008) Pittsburgh, PA MHPCD	Prospective cohort 1982 – 1985 N = 1,360 Participants recruited from prenatal clinic at Magee-Womens Hospital All women who used ≥2 Cnb joints/mo in the 1st trimester, and all women who drank ≥3 alcoholic drinks/wk in the 1st trimester were selected. After each Cnb or alcohol user was enrolled, a woman who reported using less Cnb or alcohol was selected <u>Eligibility Criteria:</u> ≥18 yrs old Live singleton births Interviewed at 6 yrs No physical or mental disability n = 648 Mean child age at assessment: 6.5 yrs Multiple regression	Children's intelligence at 6 yrs, assessed w/ the Stanford-Binet Intelligence Scale (SBIS) Fourth Edition SBIS consists of four subsets: Verbal reasoning Quantitative reasoning Abstract/visual reasoning Short-term memory Trained examiners were blind to exposure status	Maternal interviews assessing Cnb use for each mo of the 1st trimester and across 2nd and 3rd trimesters, conducted at 4th and 7th prenatal months, and delivery	Prenatal Cnb use calculated based on quantity and frequency of Cnb, hashish, and sinsemilla use To standardize the ∆-9-THC content of various forms of Cnb, quantities of hashish and sinsemilla were transformed into 3 and 2 joints, respectively Cnb exposure was quantified as average joints/day Categories of Cnb use: Abstainers Light/moderate users <1 joint/day Heavy users ≥1 joint/day	Mean IQ scores were similar for children of abstainers and light/moderate users *Regression measuring assoc btwn SBIS scores and heavy Cnb exposure during each trimester:* Coefficient (change in score assoc w/ heavy Cnb use vs. all others), R^2, p-value <u>Composite Score</u>: 1st trimester: -1.76, ns 2nd trimester: **-5.04, 0.01, p<0.05** 3rd trimester: -1.99, ns <u>Verbal Reasoning</u>: 1st trimester: **-2.63, 0.01, p<0.01** 2nd trimester: -2.85, ns 3rd trimester: -1.03, ns <u>Quantitative Reasoning</u> 1st trimester: -1.77, ns 2nd trimester: **-8.18, 0.01, p<0.01** 3rd trimester: **-5.35, 0.005**, **p<0.05** <u>Short-term Memory</u>: 1st trimester: -1.47, ns 2nd trimester: **-4.51, 0.005, p<.05** 3rd trimester: -2.38, ns <u>Abstract/visual reasoning</u> not assoc w/ prenatal Cnb A repeated measures analysis comparing the children's composite scores	Only variables that were related to SBIS w/ p<0.05 were included <u>Included:</u> Prenatal exposure to tobacco, cocaine, or alcohol Maternal cognitive ability Social support Race Number of people in household Home screen questionnaire Alcohol or drug problems of man in household No. of illnesses Maternal depression No. of siblings Poor speech, vision, hearing <u>Considered:</u> *Maternal variables:* Age at delivery Current level of education Income Work status Marital status	Children whose mothers were heavy Cnb smokers during preg had lower SBIS at 6 yrs compared to nonexposed children after controlling for covariates. Authors attribute strong assoc w/ 2nd trimester prenatal Cnb exposure to development of the endogenous cannabinoid and opioid systems in the 2nd trimester. No significant differences in prenatal substance exposure or demographic characteristics btwn included and excluded subjects The study population was equally African American and White <u>Strengths:</u> Authors note that large sample size allows for statistical power to detect even small effects of prenatal Cnb exposure on SBIS High retention rates Detailed assessment of Cnb, tobacco, and other drug use during preg Comprehensive assessment of other

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					btwn ages 3 and 6 showed that heavy Cnb use in the 3rd trimester was assoc w/ a 7.5 point decrease ($p<0.01$), compared to 3.6 points in less- or nonexposed children. The effect of prenatal Cnb exposure did not differ significantly by race.	Hostility No. of life events Current use of tobacco, cocaine, or alcohol Presence of a man in household *Child variables*: Sex Nutrition No. of injuries, hospitaliza-tions, illnesses	factors that influence cognitive development such as home, maternal, and sociodemographic characteristics <u>Limitations:</u> Authors note the use of SBIS as the only measure of cognitive functioning.

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Gold-schmidt et al. (2012) Pittsburgh, PA MHPCD	Prospective cohort Participants were recruited from prenatal clinic at Magee-Womens Hospital 1982 – 1985 N = 763 live singleton births Cohort included all women who used ≥2 Cnb joints/mo in the 1st trimester and a random sample of women who used less than this amount, and women who drank ≥3 drinks per week during the 1st trimester and a random sample of women who used less than this amount were selected n = 524 mother-child dyads seen at 14 years Attrition due mainly to loss to follow-up, refusal, moved out of the area, or child was interviewed by phone and could not be tested Multiple regression, structural equation models	<u>Primary Outcome</u>: School achievement at 14 yrs, measured by Wechsler Individual Achievement Test (WIAT). WIAT Screener has 3 subtests: basic reading, mathematics reasoning, spelling. Age-adjusted composite and subtests scores were used <u>Potential Mediators</u>: Child's cognitive development at 6 yrs, measured by the Stanford-Binet Intelligence Scale (SBIS) Childhood depressive symptoms at 10 yrs, measured by Children's Depression Inventory (CDI) Attention problems at 10 yrs, measured by the Swanson, Noland, and Pelham (SNAP) attention subscale Adolescent substance use, measured through questions adapted from Health Behavior Questionnaire	Maternal self-report Women were interviewed about 1st, 2nd, and 3rd trimester substance use in their 4th or 5th and 7th mo prenatal visit and after delivery, respectively. Women and their offspring were assessed at birth, 8 and 18 mo, and 3, 6, 10, 14, 16, and 22 yrs of age. At each phase, maternal interviews included questions about substance use, sociodemograph-ic and environmental characteristics, and psychological status	Average daily joints (ADJ) calculated from assessments of quantity and frequency of Cnb use across all three trimesters Categories: Heavy users: ADJ ≥ 1 Non-heavy: ADJ<1 Sinsemilla was counted as 2 joints of Cnb and hashish as 3 joints of Cnb	<u>Regression results</u> 1st trimester heavy Cnb exposure assoc w/ lower WIAT composite **(-2.9 points, p<0.05)** and basic reading **(-3.3 points, p<0.05)** scores <u>Structural equation models</u> Deficit in achievement mediated by effects of prenatal Cnb use on SBIS (intelligence) at 6 yrs, SNAP (attention problems) and CDI (depression) at 10 yrs, and Cnb use before age 14 Direct effects of 1st trimester Cnb exposure on WIAT score at 14 yrs, before addition of intervening mediators (standardized coefficient): 1st trimester Cnb → WIAT score (**-0.08, p<0.05**) Direct effects of 1st trimester Cnb on WIAT score at 14 yrs, after addition of 4 mediators (standardized coefficient): 1st trimester Cnb → WIAT score (-0.01, p>0.05) Total effects of 1st trimester Cnb exposure on WIAT score at 14 yrs, w/ mediators: 1st trimester Cnb → SNAP (standardized coefficient for assoc btwn Cnb and SNAP: **0.09, p<0.05**) → WIAT (standardized coefficient for assoc btwn SNAP and WIAT: **-0.11, p<0.005**) 1st trimester Cnb → adolescent Cnb use before 14 **(0.08, p<0.05)** → WIAT **(-0.05, p<0.05)**	Covariates were selected from the following, based on assoc w/ school achievement in literature and whether ss in model Prenatal _alcohol, tobacco, and other substance use Offspring gender and ethnicity Home environment Maternal SES Current maternal substance use Presence of an adult male in household Adolescent in maternal custody No. of siblings Maternal depression and hostility Maternal no. of life events Social support for mother Overall coping	The direct effects of prenatal Cnb on school achievement was ss only when mediators were not taken into account. After including IQ, attention problems, depressive symptoms, and early age of Cnb initiation, the effect was ns. The authors postulate that effects of prenatal Cnb on academic achievement is mediated by its earlier effects on these mediating factors In regression analyses, the lowest exposure group was exposed to <1 ADJ No differences in gender, BW, maternal income, or maternal education btwn participants and non-participants. Participants were more likely to be African-American and have 3rd trimester Cnb exposure Half the adolescent sample is male and 55% are African American <u>Strengths:</u> Prospective design Sample size and high retention rates increased statistical power <u>Limitations:</u> Sample consisted largely

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					1st trimester Cnb → CDI **(0.18 p<0.0005)** → WIAT → **(-0.09 p<0.05)** 1st trimester Cnb→ SBIS composite score **(-0.08, p<0.05)** → WIAT **(0.53 p<0.0005)** 1st trimester Cnb → SBIS composite score **(-0.08, p<0.05)** → CDI **(-0.16 p<0.0005)** → WIAT **(-0.09 p<0.05)** 85% of the effect of prenatal Cnb use on WIAT composite score was due to mediators Total effects of prenatal Cnb on WIAT *reading* score (standardized coefficient): **-0.1 (p<0.05);** 60% of effect was explained by mediators Mediators of WIAT reading score (standardized coefficients) SBIS (0.45, p<0.0005) SNAP (-0.11, p<0.005) CDI (-0.06, p<0.1) Cnb use before 14 (-0.06, p<0.01) Indirect effects of prenatal Cnb on WIAT reading score through mediators all ss Direct effect of prenatal Cnb on WIAT reading score ns after inclusion of mediators 2nd and 3rd trimester Cnb exposure ns assoc w/ WIAT No assoc btwn WIAT and other prenatal substance exposures	ability of mother	of low SES women, possibly limiting generalizability

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O'Connell and Fried 1991 Ottawa, Canada OPPS	Prospective cohort N not stated Participants were recruited through notices in obstetricians offices and public media and interviewed about prescription and other drug use, health history, and diet All 6-9 yo children whose mothers consumed >1 Cnb joint/wk during preg were selected (n exposed = 28) Children in control group (no maternal use of Cnb in preg) were matched w/ exposed children based on maternal tobacco and alcohol use during preg (n unexposed = 28) n = 56 The same examiner conducted the child assessments and was blind to maternal drug history Discriminant function analysis, path analysis, multivariate analysis of covariance, multiple regression	Neurobehavioral development in 6-9 yo offspring, incl. ratings of behavior problems, visual-perceptual tasks, language comprehension, and distractibility Test battery included: Global intellectual measures (WISC-R) Test of Visual Perceptual Skills Visual-motor integration (Test of Visual Motor Integration, Draw-A-Man, and Trail Making Test) Attention and memory tasks (Stroop Color and Word	Maternal self-report	Regular Cnb use (avg >1 joint/wk during preg) was compared with no Cnb use during preg Exposure ranged from 1.5 to 50 joints/wk among Cnb users	Discriminant function analysis: 7 outcome variables discriminated between the Cnb users and nonusers, explaining 36% of the group variance: Syntax Quotient Stroop Interference Visual Discrimination Visual Sequential Memory Trail Making Test-Part A Anxiety Conduct problems Effect of maternal Cnb use was evident in increased conduct problems (**F=7.39, p=0.01)** and to a lesser degree, poorer visual sequential memory (F=3.13, p=0.08) and poorer visual discrimination (F=2.89, p=0.09) After controlling for covariates, interaction of Cnb use and maternal age was assoc w/ outcomes (p=0.01), principally visual sequential memory (F(1,46)=8.69, p=0.01) and syntax quotient (F(1,46)=4.11, p=0.05): offspring of younger Cnb users scored lowest and children of younger control women scored highest on tests. The effect of Cnb use in this model was diminished (p=0.11).	Included: Aggression-external Aggression in the home Supervision Agreeableness Conscientiousness Neuroticism Mother's age at delivery Considered: Location of test HOME questionnaire variables Maternal and, paternal education Income Child's health School progress Family size Birth order Principal languages at home and school Mother's personality and intelligence Other sociodemo-graphic information	Authors note that univariate trends in this study are consistent with previous reports on young children, e.g., language comprehension scores, memory test scores, and ratings of child behavior were lower for children of Cnb users Strengths: Authors calculated 84% statistical power based on an effect size of 0.4 standard deviations Limitations: Sample size too small to permit extensive matching of subjects on potentially important confounders Authors note that the main findings were related to parental rating of child behavior problems. Whether these findings indicate true behavioral differences in children or different levels of parental tolerance is unclear. Postnatal Cnb exposure was not assessed in this study 6 women in the Cnb user group and none of the non-users reported use of other drugs in preg (all

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		Test, Knox Cube Test) Motoric assessment (Finger Tapping Test) Language comprehen-sion (Syntax Quotient derived from the Test of Language Development-Primary) Academic achievement (3 subtests of the Wide Range Achievement Test-Revised, Passage Comprehen_ sion subtest of the Woodcock Reading Mastery Test) Parent ratings of behavior problems (Conners Parent Rating Scale)			When the 6 mothers who reported other illicit drug use were excluded from analysis, the main effect of Cnb use on discriminating outcome variables was ss **(p=0.02),** as was the interaction btwn Cnb use and maternal age at delivery **(p=0.05)** Path analysis: In analysis of conduct problems, of the 6 covariates, only aggression in the home was ss (Beta weight **B=0.51, p=0.02)**. Including aggression in the home with maternal Cnb use results in a ss model (**R^2=0.28, p=0.0002)**. The combined indirect and direct effect of maternal Cnb use on conduct problems was 0.43, which approximated the zero-order correlation btwn the two variables (r=.39). No dose-response relationship was detected		infrequent, mostly cocaine).

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Richardson et al. (2002) Pittsburgh, PA MHPCD	Prospective cohort 10 yr follow up conducted btwn 1994 - 1997 N = 1360 women interviewed at 4th or 5th mo of preg 2 cohorts combined: (1) All women who drank ≥ 3 alcoholic drinks/wk and a random sample of women who drank less, (2) all women who smoked ≥ 2 marijuana (Cnb) joints/mo and a random sample who used less Cnb (women could be in both cohorts) n = 763 Eligibility criteria: live, singleton birth, available for 10-yr follow up, no condition that interfered w/ testing n = 636 (91% of eligible participants; 83% of birth cohort) n = 593 (after excluding children w/ conditions	Cognitive domains at 10 yrs of age (assessment tool) Problem solving and abstract reasoning, as well as perseverative reasoning and impulsivity (Wisconsin Card Sorting Test) Learning and memory (Wide Range Assessment of Memory and Learning (WRAML)) Mental flexibility, attention, visuomotor tracking, problem solving (Trail Making, Parts A and B, and Stroop Test) Psychomotor speed and eye-hand coordination (Grooved Pegboard) Attention and general mental efficiency, including impulsivity (Continuous Performance Test (CPT-2)) The following tests were also used: HOME-SF assessed the quality of cognitive stimulation in the home and emotional support Stanford-Binet Intelligence Scale-4th Edition assessed intellectual development Revised Children's Manifest Anxiety Scale	Maternal self-report Women were interviewed at 4th and 7th mo prenatal visit and at delivery Additional follow-up interviews and assessments occurred at 8 and 18 mos, and 3, 6, 10 yrs postpartum Women were asked about maximum, minimum, and usual amount and frequency of Cnb, hashish, and sinsemilla use Regression analyses were run separately for Cnb in each trimester and for different measures of Cnb	Interview assessed usual, maximum, and minimum frequency and quantity of Cnb, hashish, and sinsemilla Based on amount of THC in each substance: 1 hashish joint or bowl = 3 Cnb joints 1 sinsemilla = 2 Cnb joints Cnb use summarized as average daily joints (ADJ) Mothers' prenatal Cnb use (≥1 joint/day): 1st trimester =15%; 2nd and 3rd trimester = 5% *Cnb use categories: a*vg no. of joints/day (ADJ) None: no use, Light: 0<ADJ≤0.4	<u>Learning and memory (WRAML)</u>: 1st trimester heavy Cnb use ss assoc w/ lower scores on design memory **(β=−0.83)** and screening index (**β=−3.34**) Alcohol and tobacco use also were ss predictors for these subscales Attention and general mental efficiency <u>(CPT-2)</u>: 2nd trimester ADJ predicted ss more Trial 3 commission errors, a measure of impulsivity (**β=1.86**) (errors of commission appeared only toward the end of the task when the children were fatigued) Problem solving and abstract reasoning, mental flexibility, psychomotor speed and eye-hand coordination were ns assoc w/ Cnb use in any trimester The regression analyses showed that average daily alcohol use and frequency of drinking four or more drinks/day both predicted poorer performance on verbal learning and verbal and visual memory tasks Alcohol and tobacco use was also observed to affect learning and memory	<u>Included</u> Education Family income Race Child's age* Child's anxiety Child's gender HOME-SF Child's uncorrected vision problems Prenatal alcohol and tobacco use <u>Considered</u> Maternal age Male presence Work/school status Maternal depression Maternal IQ Maternal hostility Maternal life events Child's hospitalizations, illnesses, injuries No. of siblings Child's Stanford-Binet Intelligence Scale Current maternal use of alcohol,	Children who did not participate at 10 yrs were older and had fewer illnesses, and mothers who did not participate used less alcohol at 10 yrs and less Cnb in the 1st trimester. Twenty-three percent of children had repeated one or more grades. Stanford-Binet Intelligence scale composite score was 91.5 (range = 59-130). Authors stated they found that prenatal alcohol and Cnb exposure specifically affected learning and memory. Findings for prenatal Cnb exposure and increased impulsivity were consistent w/ results seen at 6 yrs. Prenatal tobacco use was also found to affect learning and memory, consistent w/ an earlier report from this project. Authors stated that the findings are particularly notable as the cohort represents children who have been exposed to light to moderate levels of drugs. <u>Strengths:</u> Detailed assessment of

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	that would interfere w/ testing) Structural equation model, t test, multiple regression Structural equation modeling was used to identify the assoc btwn exposures and neuropyschological domains	assessed anxiety-related symptoms		joints/day; Moderate: (0.4<ADJ≤ 0.89 joints/day; Heavy: ADJ>0.89joints /day Prevalence of use in 1st, 2nd, 3rd trimesters, and next 10 yrs by use category None: 58, 77, 81, 78 Light use: 19, 14, 11, 17 Moderate use: 8, 4, 3, 2 Heavy use: 14, 5, 5, 3 The majority of women reduced their Cnb use as preg progressed Dichotomous variable was constructed to examine the effect of heavy use (<0.89 vs ≥0.89 joints/day		cocaine, and tobacco Prenatal cocaine * Not included in WRAML model	Cnb use, as well alcohol,, tobacco, and other drug use during preg (by trimester) and at each follow-up phase.

Study/ Location	*Study Design, Sample Sizes, Statistical Analysis*	*Outcomes of Interest*	*Exposure Measurement Methods*	*Exposure quantification*	*Results*	*Covariates/ Confounders*	*Comments*
Rose-Jacobs et al. (2017) Boston, MA Boston City Hospital	Original cohort recruited mothers from Boston City Hospital 8-72 hours after 1990 - 1993 Participants (offspring) were high school students n = 131 <u>Eligibility Criteria:</u> GA≥ 36 wks No level III NICU care No diagnosed FAS No diagnosed HIV Mother's English fluency Mother ≥ 18 yo No documented use of illicit substances other than Cnb and cocaine No PTB No genetic syndromes Generalized estimating equation models, multivariable linear models	Executive Functioning (EF) as assessed by the Behavior Rating Inventory of Executive Functioning-Teacher Form (BRIEF-TF) every year Authors used behavioral regulation and metacognition as the primary indexes Higher scores indicate less optimal functioning Behavioral regulation refers to the ability to shift attention btwn different tasks, adaptability, and ability to regulate strong/automatic emotional responses Metacognition scores indicate higher order thinking such as the ability to understand, analyze and control one's cognitive processes in order to manage performance	<u>Intrauterine exposure:</u> Urine samples analyzed for Cnb Meconium samples analyzed for Cnb Maternal self-report Urine samples were analyzed using radio-immunoassay for benzoylecgonine, opiates, amphetamines, benzodiazepines and markers of Cnb (cannabinoids) Meconium specimens were analyzed by radio-immunoassay for the cocaine metabolite benzoylecgonine, metabolites of opiates, amphetamines, benzodiazepines, and markers of Cnb (cannabinoids) <u>Exposure in: Adolescents:</u>	<u>Intrauterine substance exposure classification:</u> Intrauterine marijuana exposure (IUME) based on a composite of urine assays, meconium assays, and maternal self-report Exposure was categorized as "unexposed" (n=97), "lighter" (n=19), "heavier" (n=15) Adolescent were considered substance-positive "if they self-reported use of alcohol, Cnb, and other substances excluding tobacco experimentation during the past 30 days or if their urine	Assoc btwn IUME and Behavioral regulation T-score as measured by multivariable linear models: β (95% CI) Heavier vs unexposed: **-11.6 (-20.8, -2.48) p = 0.01** (indicating heavier use was ss assoc w/ more optimal Behavioral Regulation) Lighter vs. unexposed: -3.64 (-10.9, 3.68) p = 0.33 Assoc btwn IUME and Metacognition T-Score as measured by multivariable linear models: β (95% CI) Heavier vs unexposed: **-11.9 (-19.91, -3.93) p = .003** Lighter vs. unexposed: -2.32 (-8.39, 3.75) p = 0.45 Authors also state that heavier IUME vs no exposure was assoc w/ significantly less metacognition clinical risk, **OR = 0.3, p = 0.04** IUTE was ss assoc w/ less optimal EF (BRIEF-TF Behavioral Regulations scores **(p<0.05)**	<u>Included:</u> Each of the other intrauterine substance exposures Maternal age Maternal race Maternal education Maternal nation of birth Child's sex BW z-score for GA Adolescent age at time of BRIEF-TF assessment Highest level of lead exposure up to 4 yo Adolescent IQ Exposure to violence <u>Considered:</u> Childhood exposure to second-hand smoke Adolescent's own substance use Any adolescent problematic substance use Primiparous vs other Neonatal HC	The cohort was recruited from an urban hospital serving a large Medicaid population. (89% African American/ Caribbean). IUME was independently assoc w/ more optimal behavior regulation and metacognition scores. This was in contrast to author's expectations and some prior studies. Authors state that explanations or mechanisms for these results "remain uncertain". Focus of the study was tobacco exposure. <u>Strengths:</u> Multiple exposure measurement methods. Substance use was determined, by both self-report and biologic measures, and analyzed for prenatal and adolescent exposure. The study was able to isolate the assoc w/ each substance independently. <u>Limitations:</u> Authors note that because of the subjective nature of the BRIEF-TF assessment, racial bias may affect the

Study/ Location	Study Design, Sample Sizes, Statistical Analysis	Outcomes of Interest	Exposure Measurement Methods	Exposure quantification	Results	Covariates/ Confounders	Comments
			adolescents voluntarily provided urine samples at each visit to screen for legal and illegal substances No-Excuse Urine test used to analyze for cannabanoids "gives a detection window of up to a wk for most drugs, and even longer for marijuana"	assay was positive" Adolescents reporting substance use prior to the past 30 days and w/ a negative urine assay were considered "ever" having used a particular substance		Household tobacco use Number of caregiver changes prior to late adolescence	predominantly African-American sample. Adolescents that dropped out of high school may have had less optimal scores but could not be included in the analysis. The focus of the paper was prenatal exposure to tobacco smoke. The number of adolescents prenatally exposed to Cnb was small.

Appendix Table 2.5 Human Neurodevelopmental Studies: Neuroimaging

Study/ Location	***Study Design, Sample Sizes, Statistical Analysis***	***Outcomes of Interest***	***Exposure Measurement Methods***	***Exposure quantification***	***Results***	***Covariates/ Confounders***	***Comments***
El Marroun et al. (2016) Rotterdam the Nether-lands Genera-tion R	Prospective cohort N not stated <u>Eligibility Criteria:</u> No significant motor or sensory disorder No moderate to severe head trauma w/ loss of consciousness No neurological disorders No claustrophobia No contraindications to MRI 3 groups of children w/ structural magnetic resonance imaging (MRI) data selected based on exposure status: Children prenatally exposed to Cnb or Cnb and tobacco Children prenatally exposed to tobacco only Children unexposed The unexposed control subjects were matched based on age and	Measures of brain morphology in 6-8 yo using MRI included: Total brain volume Cortical volume Cortical gray matter volume Cortical white matter volume Ventricular volume	Maternal self-report In 1st trimester interview, mothers indicated Cnb use before preg and were asked about current use. Information about product used and frequency of use was also collected Maternal THC levels in urine tested in early, mid, and late preg (1st available sample was used for urinalysis) Agreement between self-report Cnb use and urine analysis using Yule's Y = 0.77 indicating substantial agreement	Offspring of mothers who used Cnb at any point during preg were considered exposed (=54) Reported Cnb use during preg: mostly used it regularly-~39% weekly - ~28% monthly - 13% 3 groups were defined: 1) nonexposed controls; 2) exposed to tobacco during preg; 3) exposed to Cnb during preg	Prenatal Cnb exposure was not assoc w/ differences in total brain volume, cortical gray matter volume, cortical white matter volume, or ventricular volume Prenatal Cnb use was assoc w/ increased scores of the language domain (data were not presented) Compared to non- exposed control children, children prenatally exposed to Cnb had a thicker superior frontal area of the left hemisphere **p<0.001** and a thicker frontal pole of the right hemisphere **p<0.003** This remained ss after controlling for covariates. Similar comparisons for children prenatally exposed to tobacco showed thinner cortices in the left and right hemisphere When comparing Cnb exposed children w/ tobacco-exposed children, the differences in cortical areas were more pronounced than comparison w/ nonexposed	<u>Included:</u> Maternal [bc] education Ethnicity [bc] Household [bc] income Marital status [bc] Prenatal [b] alcohol use Maternal [bc] psycho-pathology Child's IQ BW [c] Child emotional and behavioral problems (Age and gender matched in all models)[abc] [a] = model i [b] = model II [c] = model III <u>Considered:</u> Gender Head circumference at age 6 yrs	The prefrontal cortex assoc w/ the ability to suppress responses and thoughts, attention, higher-order motor control, and working memory. Authors interpret the thicker prefrontal cortex in Cnb exposed children as altered neuro-developmental maturation <u>Strengths:</u> Prospective design minimized recall bias and establishes temporal sequence of events. Multiple exposure assessment methods. Authors note that young age of children eliminates confounding factor of Cnb smoking by offspring themselves. <u>Limitations:</u> There was no Cnb only exposure group (only 15% did not smoke tobacco during preg). Self-reported Cnb use during preg was assessed

gender to exposed children n Cnb or Cnb/Tobacco exposed = 54 n tobacco only = 96 n unexposed = 113 n total = 263 Chi square and t tests Linear regression		When comparing the subset of children exposed to both Cnb and tobacco w/ children exposed to tobacco only, there was a difference btwn clusters in the caudal middle frontal region of the left hemisphere (**p<0.001**) and the precentral region of the right hemisphere (**p <0.001)**	Gestation age Alcohol habits	only during the 1st trimester. No information about mother's postnatal Cnb use was available. Due to small sample size Cnb use was not stratified by frequency. Authors note that assessing brain morphology at one point does not allow for the inference of conclusion about the trajectory of neurodevelopment in children. Authors note that no information about maternal Cnb use in postnatal period was available. Authors note that rate of participation among Cnb exposed children was lower than for typically developing children.

Study/ Location	***Study Design, Sample Sizes, Statistical Analysis***	***Outcomes of Interest***	***Exposure Measurement Methods***	***Exposure quantification***	***Results***	***Covariates/ Confounders***	***Comments***
Smith et al. (2016) Ottawa, Canada OPPS Same population as in Smith et al. (2004 and 2006)	Prospective cohort (Preg women were recruited from 1979 - 1983) Participants were randomly selected from OPPS offspring <u>Eligibility criteria</u>: Completed the WAIS-III and C-DISC Clinical Test w/in 1 yr prior to the study 18-22 yo Right handed, No Axis I DSM0IV diagnosis No structural abnormalities English as the first language N = 35 4 participants were excluded due to current cocaine, opiates or amphetamine use (as per urine test, or self-reported use defined as ≥1x/mo)	Executive functioning Re-analysis, w/ more up-to-date methods, of data from response inhibition and visuospatial working memory tasks[1] (Smith et al., 2004, 2006), as well as 2 additional tasks[2] Tests included: "Visuospatial 2-Back" assessed visuospatial working memory[1] "Go/NoGo" assessed response inhibition[1] "Letter 2-Back" assessed working memory contrast[2] "Counting Stroop" assessed cognitive interference[2] Testing was performed while fMRI was conducted to	Prenatal exposure measurements were not specified in this article, therefore exposure measurement methods were retrieved from another OPPS article, Fried and O'Connell et al (1987): Interview during each trimester conducted in the mother's home Information collected included SES, mother's health, obstetrical history, father's medical history, 24-hr dietary recall, past and present drug use w/ emphasis on alcohol, caffeine, cigarette, and Cnb use Cnb (marijuana and hashish) use was assessed during each interview to validate consistency of	Prenatal Cnb exposure defined as regular maternal use of at least 1 joint/wk for the entire preg n = 15 non-exposed n = 16 exposed (joints/wk): Range = 0.33-54 Mean (SE) = 8.27 (3.24) Exposure was analyzed as a continuous variable Multiple independent samples t-tests were conducted at a threshold of p=0.001 uncorrected, w/ a cluster-wise correction for multiple comparisons at p = 0.05	Imaging results reported w/ T scores from T-tests determining group differences btwn the prenatally exposed and non-exposed Cnb groups All 4 executive functioning tasks identified significantly more brain activity in the prenatally exposed Cnb group compared to the non-exposed group <u>Visuospatial 2-back</u> more activity in the left posterior cingulate gyrus in Cnb-exposed group (**T= 3.90, p=0.028** uncorrected; p=0.67, corrected) <u>Go/NoGo</u> ss more activity in the left post central gyrus (**T = 4.29, p=0.003 corrected**) Similarly, in additional regions, ss more activity in the: - left precentral gyrus (**T = 3.77, p=0.003 corrected**) - left superior frontal gyrus (**T = 3.30, p=0.003 corrected**) <u>Letter 2-Back</u> ss more activity in the left middle occipital gyrus (**T = 4.03, p=0.000 corrected**) Similarly, in additional regions, ss more activity	<u>Included in T-tests:</u> Offspring current Cnb use <u>Considered for T-tests:</u> Prenatal alcohol exposure Prenatal nicotine exposure Current Cnb, nicotine and alcohol use (Performance results did not alter the imaging analyses for any task so were not used as covariates)	Measures of IQ, full scale IQ, SES, education, behavior and the Connors' Parent Rating Scale were considered and shown not to be significantly different between the groups. Current offspring drug exposures were also not different btwn the prenatally exposed and unexposed groups. There were significant differences for each task between prenatally exposed and non-exposed participants when controlling for current Cnb use. The authors noted that "consistently, the exposed group required increased neural activity in posterior brain regions to perform the tasks". Authors postulate that increased activity in the Cnb-exposed group "suggests the need for a compensatory response whereby either additional brain regions are required to perform tasks or more activity in typically activated regions is necessary". <u>Strengths:</u> Since t tests have lower power than multiple regression analyses the authors state that

Study/ Location	*Study Design, Sample Sizes, Statistical Analysis*	*Outcomes of Interest*	*Exposure Measurement Methods*	*Exposure quantification*	*Results*	*Covariates/ Confounders*	*Comments*
	n = 31 ANCOVA, t tests	assess brain activity	self-report (when inconsistent, higher figures were used)		in the: - L cerebellum **(T=3.95, p=0.000**) - right superior temporal gyrus (**T= 3.91, p=0.000 corrected**) <u>Counting Stroop</u> ss more activity in the left cuneus (**T = 3.62, p= 0.002 corrected**) In additional regions, ns more activity in the right superior frontal gyrus in Cnb-exposed group (T = 3.66, p= 0.075 corrected) No task revealed performance differences btwn the Cnb prenatally exposed and non-exposed groups for reaction times or errors of omission (Authors note that results from Smith et al. (2004, and 2006) were reported from multiple regressions rather than t-tests and are thus different from the results reported here)		the significance in t tests may be more meaningful. Use of 4 different tasks to examine executive function and related brain activity. Adjusted for current use and for multiple comparisons. <u>Limitations:</u> Authors note that the block design used is less ideal than an event-related design. Authors note the small sample size.

Study/ Location	***Study Design, Sample Sizes, Statistical Analysis***	***Outcomes of Interest***	***Exposure Measurement Methods***	***Exposure quantification***	***Results***	***Covariates/ Confounders***	***Comments***
Willford et al. (2010b) Pittsburgh, PA MHPCD	Prospective cohort Subsample of subjects recruited from MHPCD cohort based on their prenatal alcohol exposure. See Day et al (1992) for recruitment details Three groups were recruited: Offspring whose mothers who did not drink during preg Offspring whose mothers who drank in the 1st trimester only Offspring whose mothers drank throughout preg N = 585 <u>Eligibility Criteria:</u> Must be 18-22 y.o. IQ>70 No diagnosis of FAS No excessive motion in scanner n = 45	Caudate volume and asymmetry measured via MRI Increases or decreases in asymmetry might be an indication of abnormal structural and functional development	Maternal self-report at the end of each trimester	Mothers were asked about Cnb use before preg and in the 1st trimester Information was collected according to conception to recognition of conception, and recognition to confirmation of preg. A weighted average was computed from this data to create a more accurate value of 1st trimester use Prenatal Cnb exposure was measured as average daily joints (ADJ) Treated as a categorical variable: none: (ADJ = 0) moderate: (0<ADJ<1) heavy: (ADJ≥1)	Prenatal Cnb exposure was not independently, significantly assoc w/ caudate volume and asymmetry Prenatal Cnb exposure did not modify the primary effects of prenatal alcohol exposure on caudate asymmetry	<u>Included:</u> Gender Handedness Prenatal tobacco exposure Prenatal alcohol exposure	The main purpose of this study was to examine structural changes of the caudate nucleus assoc w/ prenatal alcohol consumption. Prenatal Cnb exposure was included to determine whether it had a modifying effect. There were no 2-way interactions btwn prenatal alcohol exposure, prenatal tobacco exposure, and prenatal Cnb exposure. The study included prenatal Cnb exposure as a covariate in analyzing the effects of prenatal alcohol exposure on caudate volume and asymmetry. The analysis on prenatal Cnb exposure was brief and supplementary to the research question. <u>Limitations:</u> Small sample size (n=20) for the Cnb exposed group.

Human Neurodevelopmental Studies: Neuroimaging

Studies that were not as informative as those tabulated above are summarized below.

Fried 1980 & Fried 1982

Fried (1980) and Fried (1982), from the OPPS prospective cohort study, examined the associations between in utero Cnb exposure and nervous system abnormalities in infants up to 1 year old. Pregnant women were interviewed for information about Cnb use in the year before pregnancy and each trimester, and were categorized as nonusers or irregular (≤1 joint/wk *or* exposure to secondhand Cnb smoke), moderate (2-5 joints/wk), or heavy (>5 joints/wk) users.

In Fried (1980), 89 singleton infants were assessed 60-80 hrs postpartum using the Brazelton Neonatal Assessment Scale (BNAS). Compared to infants of non- and irregular Cnb users in unadjusted analyses, infants of moderate and heavy Cnb users were:

- more likely to show alterations in visual response (chi-squared test, **p<0.01**): less likely to respond to light (chi-squared p<0.08) and habituate to light (statistics not reported)
- not different in tests of auditory responses (statistics not reported)
- less able to self-quiet (chi-squared **p< 0.02**)
- no different in being consoled by the examiner (statistics not reported)
- no different in responsiveness when held (statistics not reported)
- more apt to marked tremors (Mann-Whitney **p=0.008**)
- more apt to marked startles (Mann-Whitney **p=0.023**).

All infants of the seven Cnb users who continued to smoke >5 joints/wk during pregnancy scored ≥7 on the tremulousness scale of the BNAS, whereas 28/82 of the remaining sample scored as high (chi-squared **p<0.01**).

No differences were observed in tests of lateralization, muscular tone, hand-to-mouth behavior, general activity, alertness, or lability of states (data not reported).

Fried (1982) matched moderate to heavy Cnb users with nonusers who had comparable nicotine and alcohol habits, and adds data from assessments at 4, 9, and 30 days, and 1 year after birth. Visual stimuli responsiveness, tremors, startles, and habituation to light were similar to nonusers at 30 days. The previously reported reduced success at self-quieting was not confirmed. Findings of no differences in responsiveness when held, being consoled, laterality of handedness, general activity, and alertness were confirmed. Cnb-exposed infants were not more irritable.

Comparisons of 7 children of moderate and heavy users to children of matched non-users revealed no differences in mental, motor, or behavioral scales, and no differences in attitudes, interests, or temperament (data not reported).

Cri du chat was noted exclusively among children of Cnb users in both reports, but was not systematically recorded.

Parker et al. (1990)

Parker et al. (1990) conducted a prospective cohort study to examine the correlates and prevalence of jitteriness in full-term infants. English and Spanish-speaking women were consecutively recruited following registration for prenatal care from 1984-1987. Women were interviewed by a trained interviewer in the prenatal and immediate post-partum periods for information including use of cannabis, cigarettes, alcohol, and illicit drugs. Maternal urine samples were also obtained and tested for cocaine and cannabis metabolites. Eight to 72 hours after delivery, a pediatrician examined infants and assessed jitteriness. Of 1,054 mother/infant pairs, 259 women reported cannabis use during pregnancy, and 60 tested positive by urine assay. Neonatal jitteriness was associated with cannabis use according to **self-report (p<0.01)** and positive **urine assay (p<0.05**) (unadjusted). Cigarette and alcohol use were not associated with jitteriness, but positive urine assay for cocaine use was marginally associated with jitteriness (p=0.06).

Rivkin et al. (2008)

Rivkin et al. (2008) conducted a study of the association between prenatal exposure to cocaine use and brain volume and head circumference using volumetric MRI scans of 10-12 year old children (n = 35). Mothers of the subjects were recruited from Boston City Hospital from 1990 – 1993 in a prospective cohort study and interviewed about prenatal cannabis, alcohol, tobacco, and cocaine use. Meconium and maternal urine samples were also collected from participants following delivery. Cannabis exposure was identified either by positive self-report, or biological assay. Although the primary focus of the study was to examine any association between prenatal cocaine use and brain volume, eight of the cocaine exposed and three of the cocaine non-exposed subjects were also positive for cannabis use. Children with prenatal cannabis exposure showed a trend toward smaller mean head circumference compared to those with no exposure, in both the unadjusted and adjusted analyses; however, the difference was not statistically significant. Adjusted analyses for demographic factors and other substance use also found no statistically significant association between prenatal cannabis exposure and total brain volume, gray matter, white matter, subcortical gray matter, cerebrospinal fluid or parenchymal volume. However, the authors noted that in an analysis of variance to examine the cumulative effect of exposure to more than one substance, it was demonstrated that the smallest volumes of cerebral cortical gray

matter, total parenchymal volume, and head circumference were found in association with prenatal exposures to all 4 substances: cocaine, cigarettes, alcohol, and cannabis (cerebral cortical gray matter: 731 mL [exposed] vs 853 mL [unexposed; $P = 0.002$]; total parenchymal volume:1129 mL [exposed] vs 1287 mL [unexposed; $P = 0.006$]; head circumference: 52.3 cm [exposed] vs 55.7 cm [unexposed; $P = 0.008$]).

(total parenchymal volume = cerebral cortical gray matter + white matter + subcortical gray matter).

Appendix 3. Animal Developmental Toxicity Studies: Somatic Development

Appendix Table 3.1: in vitro and in vivo Animal Studies: Early Embryo Development and Implantation

Reference	Cell type	Concentrations/Doses	Outcomes assessed	Developmental Toxicity
Paria et al.,(1995)	Preimplantation embryos obtained from CD-1 mice on GD 2 N:cultured 5 – 20 embryos/ well	Δ^9-THC (from NIDA): 0, 6.4, 32, or 160 nM for 72 hours (h) Vehicle: Whitten's medium	Preimplantation embryo development from 2-cell to blastocyst stage	Δ^9-THC stopped embryo development (primarily between the four-cell and eight-cell stages). Data: Number of blastocysts / number of two-cell embryos at start of exposure: Control: 55/60 6.4nM Δ^9-THC: 35/58 32nM Δ^9-THC: 16/54 ($p<0.05$); 160nM Δ^9-THC: 6/54 ($p<0.001$)

Reference	Animal Model N/group	Exposure details and Timing of Evaluation	Test Agent: Doses or Concentrations	Outcomes assessed	Developmental Toxicity
Paria et al., (2001)	CD-1 pregnant mouse Wild type $CB1^{-/-}$ (CB1-) $CB2^{-/-}$ (CB2-) N= 5 – 6 /group	Subcutaneous (s.c.), miniosmotic pump under skin from GD 2 to GD 5	Δ^9-THC (from NIDA) Rate: (20µg/h) 480 µg/day (Total dose: 1.2 mg) Vehicle not specified Treatment groups: Control: (+)Δ^9-THC (inactive isomer)	Number of implantation sites on GD 5 using the blue dye method Number of embryos recovered from uterus Embryo development	#implantation site/#mice (# implantations); # blastocysts recovered Wild type: 0/5 (0); 31 blastocysts recovered CB1-XCB2-:5/6; (7.4); 4 blastocysts recovered

Reference	Animal Model N/group	Exposure details and Timing of Evaluation	Test Agent: Doses or Concentrations	Outcomes assessed	Developmental Toxicity
Paria et al., (1998)	CD-1 pregnant mice N= 4 – 13 /group	s.c., miniosmotic pump under skin from GD 2 to GD 5	Δ^9-THC (from NIDA) 4 mg/0.1 ml in propylene glycol Treatment groups: Δ^9-THC Δ^9-THC + P450 inhibitor Δ^9-THC + P450 inhibitor +CB_1R antagonist Negative control: (+)Δ^9-THC [an inactive form]	Number of implantation sites on GD 5 and 8 using the blue dye method Number of embryos recovered when the oviduct/ uterus were flushed	Animals with implantation sites (mean implantation sites) Δ^9-THC: 4/4 (11.5 ± 2); 0 blastocysts Δ^9-THC + P450 inhibitor: 1/13; (3); 91 blastocysts recovered (62 were zona-encased) Δ^9-THC + P450 inhibitor + CB_1R antagonist: 10/10 (13.2±1.3); 0 blastocysts recovered
Paria et al., (1992)	CD-1 female, ovariectomized (OVX) on PND 4 N= 6 – 8 /group	Exp.1: s.c. daily injection GD 1-6 Exp.2: Delayed implanting mice: OVX mice on GD 4, pregnancy maintained with daily injection of progesterone(P_4) from GD 5-9 GD8: Estradiol (E_2) and Δ^9-THC Exp. 3: 2 or 3 s.c. injections on GD 8 and 9	Δ^9-THC (from NIDA) Exp1: 0, or 10 mg/kg), Control: vehicle, sesame oil Exp. 2: Δ^9-THC 0. 2.5, 5, or 10 mg Δ^9-THC /kg bw on GD 8 or 9 Exp.3: Δ^9-THC 0.2, 1 and 2.5 mg/kg body wt)	Number of blastocyst implantation using the blue dye method Exp1: GD 7 Exp.2: GD 9 Exp. 3: GD 10	Exp.1: All animals (Control, 6/6 and treated, 7/7) have implantations. Number of implantation per group Control: 14.0±0.5; and Δ^9-THC:11.7 were not statistically different. Exp.2: Up to10 mg Δ^9-THC: Did not induce implantation in P-primed delayed implanting mice Did not alter E_2-induced implantation. Exp.3: 0.2, 1 or 2.5 mg Δ^9-THC /kg bw Did not induce implantation in delayed implanting mice (data not shown)

Appendix Table 3.2. Inhalation Animal Studies: Somatic Effects

Reference	Animal Model, N/group	Exposure Details, Timing of Evaluation	Test Agent, Doses or Concentrations	Outcomes Assessed	Maternal Toxicity	Developmental Toxicity
Benevenuto et al., (2017)	Mice, Balb/C N = 10/group (initially)	Nose-only, 5 min/day, GD 5.5 – 17.5 30 min isoflurane sedation for ultrasound biomicroscopy on GD 10.5 and 16.5 ½ litters eval on GD 18.5 ½ litters evaluated at birth	0.2 g cannabis containing 0.3% Δ^9THC, or filtered air	Maternal food intake, & body and uterine wt. Linear measures for 2 fetuses and placentas/ litter from ultrasound biomicroscopy. Standard litter data pre- and post-birth. No morphological assessment of soft tissue or skeletal elements. Unspecified number of fetuses/pups randomly selected for wts. of fixed organs.	↓ net wt. gain ($p = 0.03$)	Birth or fetal wt. evaluated on both a per treatment group and a per litter basis, as well as considered by sex on a per group basis. ↓birth wt. ($p < 0.02$, group, both sexes; $p < 0.001$, group, ♂only) ↑ placental wt. ($p < 0.04$, group, both sexes; $p < 0.03$, group, ♂only) ↓fetal/placental wt. ratio ($p < 0.009$, group, both sexes; $p < 0.004$, group, ♂only) Fixed wts. of lungs, brain, thymus and liver said to be significantly reduced in exposed offspring. Not analyzed on a litter basis. Unknown N.

Reference	Animal Model, N/group	Exposure Details, Timing of Evaluation	Test Agent, Doses or Concentrations	Outcomes Assessed	Maternal Toxicity	Developmental Toxicity
Rosenkrantz, 1999	Mice, Swiss-Webster N = 30-50/group	Chamber exposures to "puffs" from smoking machine. 32 sec puff exposure intervals separated by 30 sec fresh air purges. Exp GD 6-16 Eval GD 18-20	Cannabis cigarette "puffs" from an automatic smoking machine calculated to deliver total inhaled doses of Δ^9THC of: 0.8, 2.6, or 3.8 mg/kg 2 control groups: sham treated or 8 puffs of placebo smoke ("cannabinoid-extracted" cannabis plant material).	No mention of maternal outcomes Standard teratology outcomes including litter data, fetal wts., and external malformations; ~ 2/3 of fetuses eval for skeletal anomalies, 1/3 for internal soft-tissue changes.	Not mentioned	Doses combined for analysis. ↑ early resorptions/dam, total early resorptions, and total fetal mortality ($p < 0.01$)
	Rats, Fischer 344 N = 30-50/group	As above	As above	As above	As above	Doses combined for analysis. No significant effects

Reference	Animal Model, N/group	Exposure Details, Timing of Evaluation	Test Agent, Doses or Concentrations	Outcomes Assessed	Maternal Toxicity	Developmental Toxicity
Charlebois & Fried, 1980	Rats, Wistar N = 9/group 6 hrs post-birth, litters culled to 4 females and 4 males each	Exposed dams daily from 20 days premating through gestation day (GD) 20. Chamber exposures for 9 min in closed box, with 30 sec ventilations halfway through exposure period. Evaluation at birth and different postnatal days (PND), depending on the test	Cannabis smoke (CS) group: 0.6 g cannabis, containing 1% Δ^9THC, in filter-tipped cigarette tubes. 2 control groups: placebo smoke (PS) from cannabis plant material with cannabinoids removed, or no smoke (NS). For each type of exposure (CS, PS, or NS), there were three different groups each receiving a diet, containing a speciried protein level: 8, 24 or 64% protein.	Maternal wt. gain on GDs 5, 10, 15, and 19. Post-culling pup wts. on PND 1, 7, 14, 21, and 35. Acquisition of developmental landmarks: reflexes, incisor eruption, and eye opening. Neurobehavioral endpoints are discussed in section E.	↑ gestation length for CS compared to PS or NS groups on diet with 8% protein ($p < 0.002$). ↓ wt. gain GD 15-18 for all 8% protein groups regardless of cannabis exposure ($p < 0.05$).	All comparisons to NS and PS controls on corresponding % protein diet. Analysis by total group, not per litter. ↑ stillbirths for 8% protein CS group (p value not provided). 2 litters from the 8% protein CS group killed by dams on day of birth ↓ birth wt. for 8% protein diet CS pups ($p < 0.01$). ↓ pup wt. on PND 1, 7, and 14 for 8% protein CS pups ($p < 0.001$). Delayed righting reflex for 8% protein CS group (p value not provided). Delayed incisor eruption for 8 and 24%% protein diet CS pups ($p < 0.01$). Delayed eye opening and visual placing for 24% protein diet CS pups (p value not provided).

Reference	Animal Model, N/group	Exposure Details, Timing of Evaluation	Test Agent, Doses or Concentrations	Outcomes Assessed	Maternal Toxicity	Developmental Toxicity
Fried and Charlebois, 1979	Rats, Wistar N = 4/group	Whole-body exposure, 9 min/day, GD 1 – 19 Initial eval PND 0 After initial eval, litters culled to 8/dam	0.6 g cannabis containing 1.1% Δ^9THC, estimated to provide 3.3 mg/day (or 16 mg/kg-day) Δ^9THC exposure. Controls exposed to smoke from cannabis plant material with cannabinoids removed .	Daily maternal weights, and gestation length. PND 0 pups were weighed and examined for sex and external abnormalities. Litter size, sex ratio, and frequency of stillbirth were noted. Pups remaining after culling were weighed daily, and followed for appearance of upper incisors, eye opening, and pinna unfolding. Reflex acquisition and other neuro-behavioral data are discussed in section B.2.3.	None reported.	↓ total mean birth wt. for ♂($p < 0.05$) ↓ total mean birth wt. for ♀($p < 0.05$) Delayed incisor eruption and eye opening ($p < 0.01$). ↓ pup wt. gain during lactation period ($p < 0.05$). Growth caught up by PND 35.
	Rats, Wistar N = 6/group	Whole-body exposure, 9 min/day, 19 days prior to mating (♀ only). Initial eval PND 0 After initial eval, litters culled to 8/dam	As above	As above, plus time-to successful mating (evidence of vaginal plug).	None reported.	Delayed incisor eruption ($p = 0.01$ ♂; $p < 0.001$ ♀).

Reference	Animal Model, N/group	Exposure Details, Timing of Evaluation	Test Agent, Doses or Concentrations	Outcomes Assessed	Maternal Toxicity	Developmental Toxicity
	Rats, Wistar N = 8/group	Whole-body exposure, 9 min/day, 19 days prior to mating (♂only). Initial eval PND 0 After initial eval, litters culled to 8/dam	As above	As above	None reported.	↑ ratio of ♂/♀ per litter ($p < 0.05$).
	Rats, Wistar N = 6/group (initially)	Whole-body exposure, 9 min/day, 19 days prior to mating (♀ only) and through gestation. Initial eval PND 0 After initial eval, litters culled to 8/dam.	As above	As above	None reported.	No adverse effects reported.
Fried, 1976	Rats, Wistar N = 4 controls; 5 treated Pregnant rats paired by plug date, and members of pair randomly assigned to treated or control groups. 4 control and 3 treated dams delivered litters	Whole-body exposure, 9 min/day, GD 1 – 19 Initial eval PND 0 Culling and cross-fostering of half-litters: C-C, C-E, E-C, and E-E (control = C; exposed = E)	0.6 g cannabis containing 1.1% Δ^9THC, estimated 3.3 mg max total per rat. Δ^9THC exposure. Controls exposed to smoke from cannabis plant material with cannabinoids removed .	No maternal outcomes Litter size, sex ratio, stillbirths, pup wts., and external malformations noted at birth. Postnatal developmental landmarks Neurobehavioral endpoints recorded at birth and post-weaning are discussed in section B.2.3.	Not mentioned	All pups born were alive. ↓ total mean birth wt. for ♂($p < 0.005$) ↓ total mean birth wt. for ♀($p < 0.001$) E-C and E-E pups delayed incisor eruption ($p < 0.05$) E-E pups delayed eye opening ($p < 0.05$)

Appendix Table 3.3. Oral Animal Animal Studies: Somatic Effects

Reference	Animal Model, N/group	Exposure Details, Timing of Evaluation	Test Agent, Doses or Concentrations	Outcomes Assessed	Maternal Toxicity	Developmental Toxicity
Dalterio et al., (1999)	Mice Lab bred N not provided for maternal exposure experiment.	Oral, unspecified (probably gavage) For maternal exposure: Single dose on "one of the last 4 days of gestation." Eval at PND 60-80.	Δ^9THC, vehicle not specified. 0 or 50 mg/kg,	Testes and seminal vesicle wts. of adult ♂.	Not noted	No effect on testes or seminal vesicle wts. of adult ♂offspring. No data or analysis presented for any outcome.
	Mice (lab bred) N = 18 adult ♂/group for paternal exposure experiment.	Oral, unspecified (probably gavage) For paternal exposure: 3X/wk for 5 wks. Mated to produce F1. Eval: Litters sired by F1 ♂offspring.	Δ^9THC, vehicle not specified. 0 or 50 mg/kg.	Untreated ♀s mated to F1 ♂ offspring of Δ^9THC -treated ♂s were sacrificed between GD 15-19. Numbers of corpora lutea, resorptions, and living or dead fetuses were assessed.	Not noted	36% of F1 ♂offspring of Δ^9THC -treated ♂s could not produce a single F2 litter. 2 litters sired by F1 ♂ offspring of Δ^9THC-treated ♂each had 1 pup with severe malformations. No data or analysis presented for any outcome.
Bonnin et al., (1995)	Rats, Wistar N not provided	Route, unspecified. We believe it was oral gavage due to sesame oil vehicle. Daily from GD 5 through eval on GD 14 or GD 16	Δ^9THC, 0 or 5 mg/kg-day. Controls: sesame oil vehicle only.	Maternal food intake, water intake, and wt. gain. Litter size Placental and fetal wt. on GD 14 and GD 16 Neurobehavioral endpoints are discussed in section B.2.3.	None reported	↓ placental wt. on GD 16 ↑fetal wt. on GD 14 and ↓on GD 16 For all above: $p < 0.05$. "Values [analyzed] are means ± SEM of more than 10 determinations *per* group."

Reference	Animal Model, N/group	Exposure Details, Timing of Evaluation	Test Agent, Doses or Concentrations	Outcomes Assessed	Maternal Toxicity	Developmental Toxicity
Abel et al., (1990a)	Long Evans rats Pregnant ♀ of different ages: 3 mo (n= 53) 4 mo (n= 38) 6 mo (n=37)	Oral, "Intubated" (presumed gavage) Daily from GD 6 to parturition. Animals in 0 and 10 mg/kg groups were pair fed to those given the 25 mg/kg dose.	THC (Δ^9 not specified), 0, 10 or 25 mg/kg-day Controls: sesame oil vehicle.	Maternal wt. gain Litter size, birth wt., PND 21 wt., postnatal mortality. Neurobehavioral endpoints are discussed in**Section 0**	↓ maternal wt. gain with increasing maternal age combined with increasing THC exposure ($p < 0.05$)	↓ birth wt. with increasing maternal age combined with increasing THC exposure ($p < 0.05$)
Abel et al., (1990b)	Rats, Long Evans N = 10	Oral, "Intubated" (presumed gavage) Daily from GD 6 through parturition.	Δ^9THC: 0, 25, 50 mg/kg-day Controls: sesame oil vehicle.	Maternal wt. gain. Litter size, birth wt.	↓ maternal wt. gain at 25 mg THC/kg-day ($p < 0.01$).	↓ birth wt. trend over both doses of THC ($p < 0.02$).

Reference	Animal Model, N/group	Exposure Details, Timing of Evaluation	Test Agent, Doses or Concentrations	Outcomes Assessed	Maternal Toxicity	Developmental Toxicity
Hutchings et al., (1987)	Rats, Wistar N = 17-20/group	Oral gavage, daily GD 8-22 On day of birth, all pups fostered to untreated dams and followed until PND 32. All control and experimental dams sacrificed for evaluation after delivery.	Δ^9THC in sesame oil vehicle. 0, 15, 50 mg/kg-day. 2 control groups: not treated (NT), and a group pair fed (PF) to the 50 mg/kg-day group. PF animals were given vehicle.	Dams sacrificed for evaluation following parturition. Wt. gain, implantation sites, and resorptions. Newborn pups assessed for viability, sex, and wt.. Wt. gain from birth to PND 32.	No maternal deaths. ↓ wt. gain with 15 or 50 mg Δ^9THC/kg-day, , or PF groups compared to NT controls ($p < 0.001$). 75-80% ↓ in food and water consumption following 1st dose of 50 mg Δ^9THC/kg-day on GD 8. Gradual recovery by GD 12-13.	↑ perinatal mortality for both treated groups vs either NT or PF controls ($p < 0.05$). ↑ postnatal mortality: both treated groups vs NT or PF ($p < 0.005$). ↓ birth wt. for both treated groups vs NT group ($p < 0.001$). ↑ proportion of ♂pups for both treated groups vs NT group ($p < 0.001$). By PND 11, the 15 mg/kg group had caught up in wt. to NT controls, but the 50 mg/kg group still weighed less ($p < 0.05$). By PND 32 there were no significant differences among groups in wt..
Dalterio and Rooij, (1986)	Mice (lab bred) N = 8 pregnant ♀/group; 7-10 ♂ offspring tested	Oral, gavage; single treatment on GD 12 Eval of ♂offspring at maturity	Δ^9THC in sesame oil, 0, 50 mg/kg Controls: sesame oil only	Adult ♂offspring mated, tested for fertility; plasma testosterone (T), LH and FSH; testes wt.; testicular histology for sperm evaluations.	Not noted	↓ plasma T and LH ($p < 0.05$).

Reference	Animal Model, N/group	Exposure Details, Timing of Evaluation	Test Agent, Doses or Concentrations	Outcomes Assessed	Maternal Toxicity	Developmental Toxicity
Dalterio et al., (1986a)	Mice (lab bred) N = 5 – 6: ♂s at evaluation	Oral gavage, single treatment on GD 12. Eval: adult ♂offspring sacrificed 24 hrs following surgical castration. Castrated ♂s given 20 µg crystalline T subcutaneously (s.c.) at 1 hr. prior to assessment.	Δ^9THC in sesame oil, 0, 50 mg/kg Controls: sesame oil only	bw, liver wt., liver concentration of cytochrome P-450, and plasma testosterone concentration.	Not noted	↑ liver wt. and liver cytochrome P-450 concentration following prenatal THC exposure. ↓ plasma T at 1 hr following s.c. injection with 20 µg crystalline T. (Findings above stated to be statistically significant, but p values not provided).
Dalterio et al., (1986b)	Mice (lab bred) N = 10 pregnant ♀/group 3-4 ♂ offspring/litter evaluated.	Oral gavage, single treatment on GD 12. Litters delivered. On PND 21, ♂weaned and housed in groups of 3-4/cage. Eval: adult ♂offspring killed 2 wks following surgical castration.	Δ^9THC in sesame oil, 0, 50 mg/kg Controls: sesame oil Some (unspecified N) castrated ♂s given 20 µg crystalline T s.c. at 1 hr. prior to blood obtained by cardiac puncture.	bw, liver wt., serum gonadotropins and liver cytochrome P-450 measured.	Not noted	↑ liver wt. and liver cytochrome P-450 concentrations ($p < 0.05$) Following prenatal treatment with Δ^9THC, castrated males not given a challenge dose of T had ↓ plasma LH ($p < 0.05$) compared to controls.

Reference	Animal Model, N/group	Exposure Details, Timing of Evaluation	Test Agent, Doses or Concentrations	Outcomes Assessed	Maternal Toxicity	Developmental Toxicity
Abel et al., (1984)	Rats, Long-Evans N = 12-22	"Intubation" (presumed oral, gavage) Initial set of doses given on GD 1-5 Dosing continued with higher doses through GD 21 Pair fed Control (to 150 mg/kg group) and Ad lib Untreated control	Δ^9-THC in olive oil Initial set of doses: 0, 5, 10, 20, 40, 50 mg/kg-day Same animals then dosed with 0, 50, 150 mg/kg-day Vehicle: olive oil Controls: Untreated, ad lib fed Vehicle controls (and animals given 50 mg/kg-day) pair fed and watered to animals given 150 mg/kg-day	Pregnancy frequency at term/confirmed matings Gestation length Maternal wt. gain, absolute and net Pup viability at birth, and total litter size (live + dead). Pup wt. at birth (litter wt./number pups), after culling, PND 7 and PND 21 by group.	↓ pregnancies at term ↓ maternal wt. gain, both absolute and net ($p < 0.001$ for both doses compared to both control groups)	↓ pregnancies at term: 10/22 at 50 mg/kg-day 2/22 at 150 mg/kg-day ($p < 0.001$, for both doses compared to both control groups) ↓ pup wt. at birth, after culling, and on PND 7 ($p < 0.001$ for both doses, both time points, compared to both control groups).

Reference	Animal Model, N/group	Exposure Details, Timing of Evaluation	Test Agent, Doses or Concentrations	Outcomes Assessed	Maternal Toxicity	Developmental Toxicity
Dalterio et al., (1984)	Mice (lab bred) N ♂s at evaluation = 3 - 15/group	Oral, gavage; single treatment on GD 18. Pups delivered normally on GD 19-21; litters culled to 5-6 ♂ pups/litter. Half of ♂s from each group castrated at 2 weeks prior to sacrifice Eval: PND 60-80	Δ^9THC in sesame oil, 0, 50 mg/kg Controls: sesame oil only	Measurement: Plasma T, LH, and FSH. Testicular T, testes wt., seminal vesicle wt. In vitro culture of decapsulated testes from adult ♂s prenatally exposed to Δ^9THC. Testes wt. and T level following 4 hr incubation with 12.5 mlU human chorionic gonadotropin (hCG). Experiments on neuroendocrine function are discussed in section B.2.3.	Not noted	No significant changes in hormone levels with Δ^9THC. Plasma T and FSH ↓ in prenatally Δ^9THC-exposed and later castrated ♂at 1 hr following injection of 20 µg exogenous T ($p < 0.05$). ↑ response to intratesticular injection of 10 ng LH in adult ♂ prenatally exposed to Δ^9THC ($p < 0.05$). ↑ wt. of decapsulated testes from adult ♂ prenatally exposed to Δ^9THC following 4 hr culture with 12.5 mlU hCG compared to vehicle controls ($p < 0.05$).
Dalterio and Bartke, (1981)	Mice (lab bred) N not specified	Oral, gavage; daily on f GD 12-16 Eval: GD 16 (4 hr following final dose)	Δ^9THC in sesame oil vehicle. 0, 15, 50 mg/kg-day. Controls: sesame oil only	Fetal viability and wt., ano-genital distance, total fetal T and dihydrotestosterone (pg/g bw).	Not noted	↑ fetal deaths ($p < 0.05$) ↓ T and dihydrotestosterone in ♂ fetuses ($p < 0.05$).

Reference	Animal Model, N/group	Exposure Details, Timing of Evaluation	Test Agent, Doses or Concentrations	Outcomes Assessed	Maternal Toxicity	Developmental Toxicity
Fleischman et al., (1980)	Rats, Fischer 344 N = 50/group (approx. 10/interval sacrifice/dose)	Oral, gavage, daily on GD 6-15 Sacrifice for eval on each of GD 8, 11, 14, 17, or 19 (after 2, 5, 8, or 10 doses, respectively)	"Pure synthetic" Δ^9THC in sesame oil, 0,12.5, 25, 50 mg/kg-day 2 control groups: Sham treated, or sesame oil only	Dam bw, uterine wt., location of implantation sites, "placental condition," number of live and dead fetuses.	Reversible central nervous system (CNS)-inhibition; observed for a day or two of treatment. No effects on rates of wt. gain.	↓ live fetuses/litter in all treated groups ($p < 0.05$; calculated by OEHHA. ↑ resorptions in all treated groups. ↑ whole litter resorptions among treated dams ($p < 0.05$; calculated by OEHHA).
	Mice, CD-1 N = 90/group (approx. 10/interval sacrifice/dose)	Oral, gavage, daily on f GD 6-15 Sacrifice for eval on each of GD 8, 11, 14, 17, or 19 (after 2, 5, 8, or 10 doses, respectively)	"Pure" synthetic Δ^9THC in sesame oil. 0, 150, 300, 600 mg/kg-day. 2 control groups: Sham treated, or Sesame oil only	As above	No effects on behavior. No effects on rates of wt. gain.	↑ fetal mortality in all treated groups (% total). ↑ whole litter resorptions among treated dams. No statistics reported.
	Rats, Fischer 344 N = 20-23/group	Oral, gavage, daily on GD 5-7, 6-8, 7-9, 8-10, or 9-11. Sacrifice for eval on GD 14	"Pure" synthetic Δ^9THC in sesame oil. 0, 50 mg/kg-day. Control group: Sesame oil only	Fetal viability and mortality.	Reversible CNS-inhibition; observed for a day or two of treatment. No effects on rates of wt. gain.	↓ live fetuses/litter with treatment on GD 7-9, 8-10, or 9-11 ($p < 0.01$). ↑ fetal mortality (% total) with treatment on GD 7-9 ($p < 0.01$) or GD 8-10 ($p < 0.05$).

Reference	Animal Model, N/group	Exposure Details, Timing of Evaluation	Test Agent, Doses or Concentrations	Outcomes Assessed	Maternal Toxicity	Developmental Toxicity
	Rats, Fischer 344 N = 12-15/group	Oral, gavage, daily on GD 6-9 All dams laparotomized on GD 6 (before treatment), numbers and locations of implantation sites recorded. Evaluated at GD 12 or GD 16	"Pure" synthetic Δ^9THC in sesame oil. 0, 50 mg/kg-day. 2 control groups: Sham treated, or Sesame oil only	Numbers of embryos before and after treatment. % whole litter resorption and fetal mortality, based on embryo counts at laparotomy.	Reversible CNS-inhibition; observed for a day or two of treatment. No effects on rates of wt. gain.	↑ whole litter resorption at both sacrifice days ($p < 0.003$) ↑ fetal mortality (implants at laparotomy vs. fetuses at necropsy) on GD 12 ($p < 0.01$) and GD 16 ($p < 0.001$).
Luthra, Y 1979	Rats, Fischer N = 10/dose group	Oral, "intubation" (presumably gavage) Group 1: treated throughout gestation, evaluated within 24 hrs of birth. Group 2: treated throughout gestation, evaluated at PND 7	Δ^9THC (96% pure) in sesame oil at 0, 1, 5, or 10 mg/kg-day Controls: sesame oil only	Maternal animals: body and organ wts. Pups: examined for viability and external malformations	No adverse effects reported.	No adverse effects reported.

Reference	Animal Model, N/group	Exposure Details, Timing of Evaluation	Test Agent, Doses or Concentrations	Outcomes Assessed	Maternal Toxicity	Developmental Toxicity
Joneja, 1977	Hamsters, Golden Syrian N = 5-10/group	Oral, gavage, single dosing on one of GD 7-12 Eval on GD 15	Δ^9THC (96% pure) in olive oil. Single doses of 0, 125, 250, or 500 mg/kg. Control group: olive oil only.	Number of implantations, viability, fetal wt., external and skeletal abnormalities. Data not presented on per litter basis.	Not noted.	↓ fetal wt.: GD 7; 125 and 500 mg/kg GD 8; 250 and 500 mg/kg GD 9, 10, 11, and 12; all doses (all $p < 0.05$) GD 10, 500 mg/kg 3/56 live fetuses (2 from one litter, 1 from another litter) had "twisted hind limbs" (gyropodia). Sig ↑ in external malformation frequency ($p < 0.05$).
	Hamsters, Golden Syrian N = 10-13/group	Oral, gavage, daily on GD 7-10, 9-12, or 7-12. Eval on GD 15	Δ^9THC (96% pure) in olive oil. Single doses of 0, 25, 50 or 100 mg/kg-day. Control group: olive oil only.	Number of implantations, viability, fetal wt., external and skeletal abnormalities. Data not presented on per litter basis.	Not noted.	↓ fetal wt.: GD 7-10 and 7-12; all doses GD 9-12; 100 mg/kg-day (all $p < 0.05$) GD 7-10; 4/96 live fetuses. Sig ↑ in external malformation frequency ($p < 0.05$)

Reference	Animal Model, N/group	Exposure Details, Timing of Evaluation	Test Agent, Doses or Concentrations	Outcomes Assessed	Maternal Toxicity	Developmental Toxicity
Joneja, 1976	Mice, Swiss Webster N = 8-14/group	Oral, gavage, single dosing on one of GD 7-11. Evaluated on GD 18.	Δ^9THC (96% pure) in olive oil. Single dose of 0, 100, 200, or 400 mg/kg. Control group: olive oil only.	Number of implantations, viability, fetal wt., external and skeletal abnormalities. Data not presented on per litter basis	Not noted.	↓ fetal wt.: GD 7; 400 mg/kg GD 8, GD 10; all doses GD 9, GD 11: 200 and 400 mg/kg (all $p < 0.05$) Exencephaly: 11/91 live fetuses with exencephaly at 400 mg/kg on GD 9. No cases among controls or at 100 mg/kg. 1/129 cases at 200 mg/kg.
	Mice, DBA/2J N = 8-15/group	Oral, gavage, single dosing on one of GD 7-11. Evaluation on GD 18.	Δ^9THC (96% pure) in olive oil. Single dose of 0, 100, 200, or 400 mg/kg-day. Control group: olive oil only.	Number of implantations, viability, fetal wt., external and skeletal abnormalities. Data not presented on per litter basis	Not noted.	↓ fetal wt.: GD 8; all doses GD 9; 400 mg/kg GD 10; 200 mg/kg GD 11; 200 and 400 mg/kg (all $p < 0.05$)

Reference	Animal Model, N/group	Exposure Details, Timing of Evaluation	Test Agent, Doses or Concentrations	Outcomes Assessed	Maternal Toxicity	Developmental Toxicity
Wright et al., (1976)	Rats, Charles River N = 10 ♂and 20 ♀/group	Oral, gavage ♀ daily from 14 days prior to mating until 21 days post-partum. ♂daily from 40 days of age through mating. Evaluation: half of ♀ sacrificed on GD 14, remainder followed through delivery and weaning.	Synthetic Δ^9THC (97% pure) in sesame oil, 0.05 or 0.5% solutions. Doses: 0, 1.5, 5.0 mg/kg-day. Controls: sesame oil only	On GD 14: number pregnant, corpora lutea, implantation sites, resorption sites, viable fetuses, fetal body wt. At birth: numbers of viable and dead fetuses/litter	Mention of effects on bw (♀ & ♂) and behavioral symptoms, but data and analysis not presented.	On GD 14: no significant effects. At birth: no significant effects.
	Rats, Charles River N = 40 controls, 20-30/treated group	Oral, gavage; daily GD 15 – PND 21 Cross-fostering at birth created dose group exposed prenatally only, followed postnatally. Eval: Birth, PND 1, 4, 12, and 21.	Synthetic Δ^9THC (97% pure) in sesame oil, 0.05 or 0.5% solutions. Doses for prenatal-only exposure: 0, 5.0 mg/kg-day. Control group: sesame oil only	Numbers and wt. of offspring recorded at birth and PND 1, 4, 12, and 21. All litters culled to a max of 10 pups on PND 4.	Not noted.	With prenatal-only exposure to 5.0 mg/kg-day no effects observed until PND 21. On PND 21: ↑ pup bw ($p < 0.01$) ↓ sex ratio ($p < 0.05$)
	Rats, Charles River N = 10 controls, 20/treated group.	Oral, gavage, daily on GD 6-15. Eval: GD 20	Synthetic Δ^9THC (97% pure) in sesame oil, 0.05 or 0.5% solutions. Doses: 0, 5.0, 15, 50 mg/kg-day. Control group: sesame oil only	Numbers of corpora lutea, implantations, resorptions, live fetuses. Fetal sex and wt.; external, internal, and skeletal anomalies.	↓ wt. gain: 70% of controls in 5 mg/kg-day dose group 50% of controls in 15 and 50 mg/kg-day dose groups ↑ behavioral symptoms with ↑ dose.	No adverse effects attributable to treatment.

Reference	Animal Model, N/group	Exposure Details, Timing of Evaluation	Test Agent, Doses or Concentrations	Outcomes Assessed	Maternal Toxicity	Developmental Toxicity
	Rabbits, New Zealand N = 44 vehicle controls, 27 positive controls, 14-17/treated group.	Oral, gavage, daily on GD 6-18 Eval: GD 29	Synthetic Δ^9THC (97% pure) in sesame oil, 0.05 or 0.5% solutions. Doses: 0, 1.5, 5.0 mg/kg-day. Control groups: vehicle - sesame oil only; positive control - thalidomide at 37.5 mg/kg-day.	Numbers of corpora lutea, implantation sites, resorptions, aborted fetuses, viable fetuses, fetal wt., Living fetuses incubated 24 hr and observed for survival. External, visceral, and skeletal anomalies (not presented on a litter basis)	"Slight" ↓ in wt. gain with all doses. Slight, temporary, sedation at 5 mg/kg-day.	↓ implantation sites/litter ($p < 0.05$). ↓ viable fetuses/litter ($p < 0.01$).
Vardaris et al., (1976)	Rats, Sprague-Dawley N = 8/group Litters culled to 3 ♂and 3 ♀ each.	Oral, gavage, daily from GD 3 – parturition	Δ^9THC as an aqueous suspension in polyvinylpyrrolidone (PVP) Doses: 0, 2 mg/kg-day Controls: vehicle only	Dams and pups weighed daily	Number litters at birth: 5/8 controls 7/8 treated	No "reliable weight differences between the 2 groups of pups." 1 treated litter had 3 stillborn pups and 7 healthy pups. Mean size for treated litters was 11.7, compared to 8.4 for controls.
Fleischman et al., (1975)	Mice, CD1 N = 22-40/group	Oral, gavage, daily on each of GD 6-15 Evaluated "24 – 36 hours before expected delivery."	Δ^9THC in sesame oil. 0, 5, 15, 50, 150 mg/kg-day. Control group: sesame oil only	Number and location of implantations, number live fetuses, sex, fetal wt., and external abnormalities. 2/3 fixed for skeletal evaluation; 1/3 for internal evaluation.	No treatment-associated maternal mortality or effects on wt. gain.	No significant findings; data not presented per litter.

Reference	Animal Model, N/group	Exposure Details, Timing of Evaluation	Test Agent, Doses or Concentrations	Outcomes Assessed	Maternal Toxicity	Developmental Toxicity
Grilly et al., (1974)	Chimpanzees N exposed = 5♂, 3♀ N unexposed = 1 ♂, 4 ♀ 2 matings btwn exposed ♂and ♀ 1 mating of exposed ♀ to unexposed ♂ 4 matings of exposed ♂to unexposed ♀ (1 pair mated twice)	Oral, unspecified 50-150 doses at unspecified intervals, from 1.5 months – 5 years prior to mating. No treatment during mating or gestation.	Synthetic Δ^9THC, or "a marijuana extract distillate." 1 – 2.1 mg/kg-dose Compared to reproductive histories of untreated colony animals.	Pregnancy outcome	Not noted	2 ♂offspring born to treated couples. 1 set of ♀ twins born to a treated ♀ mated to an untreated male. 3 ♂offspring and 1 fetal death resulting from treated ♂s mated to untreated ♀s. 77 colony births to untreated pairs resulted in 59 normal births, 13 fetal deaths, and 5 "other" offspring deaths.

Appendix Table 3.5. Injection Animal Studies: Somatic Effects

Reference	Animal Model, N/group	Exposure Details, Timing of Evaluation	Test Agent, Doses or Concentrations	Outcomes Assessed	Maternal Toxicity	Developmental Toxicity
Silva et al., (2012)	Rats, Sprague Dawley N = 12 control and 9 treated.	Injection, intravenous (i.v.), via a surgically-implanted permanent intravenous catheter. Daily injections prior to mating, continued on GD 1-21. Evaluated at birth	Δ^9THC in pluronic acid in saline. Dose: 0, 1.15 mg/kg-day. Control group: vehicle only	Maternal wt. recorded weekly from GD1-22. Gestation length, number liveborn pups, and sex ratio. Neurobehavioral endpoints recorded postnatally are discussed in section B.2.3.	No significant effects on maternal wt. gain.	No significant effects on pup birth wt., live litter size, or sex ratio.

Reference	Animal Model, N/group	Exposure Details, Timing of Evaluation	Test Agent, Doses or Concentrations	Outcomes Assessed	Maternal Toxicity	Developmental Toxicity
Wenger et al, (1997)	Rats, Sprague–Dawley N= 16-17 (initial pregnant)	Injection, i.p. Daily from GD 14 until delivery Eval: PND 0, 5, 10, 15, 20.	Δ^9THC in propylene glycol. Doses: 0, 0.02 mg/kg-day	Gestation length, litter size, stillbirths/litter, sex ratio. Body and pituitary wts. on PND 0, 5, 10, 15, 20. Hormonal assays on same PND days as above: gonadotropin releasing hormone (GnRH), luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin (PRL) and growth hormone (GH).	No change in behavior of pregnant rats was observed.	↑ stillbirths/litter: 0.05 for controls; 2.53 for exposed (no statistical analysis). ↓ pup wt. for ♂pups only, on PND 0 and PND 5 ($p < 0.05$; by group). ↓ pituitary wt. both sexes on PND 0 and PND 5 ($p < 0.02$ or $p < 0.01$; by grp). ↑ GnRH in ♂on PND 0 ($p < 0.01$); ♀ on PND 0, 5, 15 ($p < 0.01$ or $p < 0.02$). ↓ pituitary and serum LH in ♂& ♀ on PND 0 ($p < 0.05$ or $p< 0.01$ or $p < 0.02$) ↑ serum LH in ♀ on PND 5 ($p < 0.05$) ↑ pituitary PRL in ♂on PND 0 ($p < 0.005$) ↓ pituitary PRL in ♀ on PND 0 ($p < 0.01$) ↑ pituitary PRL in ♀ on PND 5 ($p < 0.01$) ↓ serum PRL in ♂and ♀ on PND 0, and in ♀ on PND 5 ($p < 0.01$) ↓ pituitary GH in ♂& ♀ on PND 0 & PND 5 ($p < 0.01$, $p < 0.02$, or $p < 0.005$) ↓ serum GH in ♂and ♀ on PND 0, and in ♂on PND 5 ($p < 0.05$ or $p < 0.01$)

Reference	Animal Model, N/group	Exposure Details, Timing of Evaluation	Test Agent, Doses or Concentrations	Outcomes Assessed	Maternal Toxicity	Developmental Toxicity
Asch et al., (1986)	Monkeys, rhesus N = 5/group	Injection, intramuscular (i.m.) Group 1: daily from mating through term. Group 2: GD 55-109 Delivery by C-section on GD 154-157	Δ^9THC, suspended in sterile saline using Emulphor. Doses: 0, 2.5 mg/kg-day Controls: vehicle only	Mothers observed and any vaginal bleeding recorded. Fetal growth evaluated by ultrasonography at 4-5 weeks gestation, weekly thereafter (mothers given ketamine hydrochloride at 5-7 mg/kg for the procedure. Hormone levels assayed in maternal blood samples..	Δ^9THC associated with "disruptive effects on the hormones during pregnancy"	Group 1: 4/5 pregnancies lost in treated animals (3 early abortions and 1 stillbirth). Controls 5/5 live births. Group 2: 1 early infant death; 4/5 live born. Controls 5/5 liveborn.
Sofia et al., (1979)	Rabbits, New Zealand White N = 9-13/group	Injection, s.c. Daily on GD 7-19. Eval: GD 29	Δ^9THC in propylene glycol. Doses: 0, 15, 30 60 mg/kg-day Control groups: vehicle only, and positive control oral thalidomide at 200 mg/kg-day.	Maternal behavior and clinical signs, food consumption, and body wt. throughout gestation. Corpora lutea, implantations, resorptions, fetal viability and sex, mean litter weight, external, internal and skeletal anomalies. Live fetuses incubated for 3 hours and checked again for viability.	Maternal mortality: 2/11, 30 mg/kg-d; 1/10 at 60 mg/kg-d (0/13 for vehicle controls and 0/9 at 15 mg/kg-day). ↓ bw on GD 13, 19, and 29 with 15 or 60 mg/kg-day. ↓ bw on GD 13 and 19 with 30 mg/kg-day. (all at $p < 0.05$ compared to vehicle controls) ↓ food intake on GD 7-13 and 14-20 at all doses ($p < 0.01$ or 0.001)	↓ live fetuses/litter with 30 or 60 mg/kg-day. ($p < 0.02$ and $p < 0.05$, respectively). ↓ fetal wt. (total group) with all doses (all at $p < 0.001$). ↑ sex ratio with 30 mg/kg-day ($p < 0.05$) ↓ survival at 3 hours ($p < 0.05$).

Reference	Animal Model, N/group	Exposure Details, Timing of Evaluation	Test Agent, Doses or Concentrations	Outcomes Assessed	Maternal Toxicity	Developmental Toxicity
Harbison et al., (1977)	Mice, Swiss origin N = 8-15/group	Injection, intraperitoneal (i.p,) on GDs 8 & 9, 10 & 11, or 12 & 13. Eval on GD 19	THC, 97% Δ^9 and 3% Δ^8 suspended in 10% Tween-80 and saline. 0, 50, 200 mg/kg Control group: vehicle only Some group pretreated with phenobarbital or 2-diethylaminoethyl-2,2-diphenyl-valerate).	Number and uterine position of live, dead, and resorbed fetuses. External anomalies, internal and skeletal anomalies.	Not mentioned.	↑ resorption rate with 50 mg/kg on GD 8 & 9 ($p < 0.05$). ↑ resorption rate with 200 mg/kg on GD 8 & 9 or GD 12 & 13 ($p < 0.05$). ↓ fetal bw with 50 or 200 mg/kg on GD 10 & 11 or 12 & 13 ($p < 0.05$). Results for pre-treated groups are less relevant to this evaluation and are not described here..
Joneja, (1976)	Mice, Swiss Webster N = 9-11/group	Injection, i.v., single dosing on one of GD 7-11. Evaluation on GD 18	Δ^9THC (96% pure) in 10% Tween 80and saline. Single dose of 0, 10, or 20 mg/kg. Vehicle control	Number of implantations, viability, fetal wt., external and skeletal abnormalities.	Not noted.	↓ fetal wt.: GD 7, GD 9, GD 10; both doses (all $p < 0.05$). Data not presented on per litter basis
	Mice, Swiss Webster N = 5-12/group	Injection, s.c., single dosing on one of GD 7-11. Evaluation on GD 18.	Δ^9THC (96% pure) in olive oil. Single dose of 0, 3.0, 4.5, 6.25, 12.5, 25.0, 50.0, 75.0, 150.0, 300.0 mg/kg Control group: vehicle only	Number of implantations, viability, fetal wt., external and skeletal abnormalities.	Not noted.	↓ fetal wt.: GD 8; 4.5, 6.25, 25.0, 75.0, and 300.0 mg/kg GD 9; 50.0 and 75.0 mg/kg. GD 10; 6.25, 12.5, 25.0, 50.0, and 75.0 mg/kg. GD 11; 6.25, 12.5, 25.0, 50.0, 75.0, and 300 mg/kg. (all $p < 0.05$) Data not presented on per litter basis

Reference	Animal Model, N/group	Exposure Details, Timing of Evaluation	Test Agent, Doses or Concentrations	Outcomes Assessed	Maternal Toxicity	Developmental Toxicity
Mantilla-Plata et al., (1976a)	Mice, Swiss Webster N = 8-12/group	Injection, i.p, daily on GD 8-13, 8-9, 10-11, or 12-13. Eval on GD 19	Δ^9THC in 10% Tween 80 and saline. Doses: 0, 50, 200 mg/kg-day. Control group: vehicle only	Standard litter data, fetal examinations for external, internal, and skeletal anomalies.	Not noted	↑ resorptions: At 50 mg/kg-day on GD 8-13, 8-9, or 10-11. At 200 mg/kg-day on GD 8-9, 10-11, or 12-13. ↓ fetal bw: At 50 mg/kg-day on GD 10-11 or 12-13. (all above at $p < 0.05$; analyzed per group not per litter)
Mantilla-Plata et al., (1976b)	Mice, Swiss ICR or CFW N = 8-15/group	Injection, i.p, daily on GD 10-11 or 12-13. Eval on GD 19	Δ^9THC (97% pure) in 10% Tween-80 Doses: 0, 50, 200 mg/kg-day. Control group: vehicle only .	Maternal bw recorded on days of treatment and at sacrifice. Standard litter data, fetal examinations for external, internal, and skeletal anomalies.	Not noted.	↑ resorptions: At 50 mg/kg-day on GD 10-11. At 200 mg/kg-day on GD 10-11 or 12-13. ↓ fetal wt. at both doses on GD 10-11 or 12-13 (all above at $p < 0.05$; analyzed per group not per litter)
Banerjee et al., (1975)	Rats, Charles River CD rats of Sprague-Dawley origin N = 10/group	Injection, sc, daily on each of GD 6-15 Eval on GD 20	Δ^9THC in propylene glycol Doses: 0, 25, 50, 100 mg/kg-day 2 control groups: untreated, or propylene glycol vehicle	Maternal outcomes not specified, but results note wt. and behavioral changes. Standard litter data, fetal examinations for external, internal, and skeletal anomalies.	↓ wt. gain said to be significant, but not dose-related (graph presented without statistics). ↓ locomotor activity and ↑ sensitivity to stimuli noted, but not quantified.	Data analyzed by total fetuses/group, rather than per litter. ↓ mean fetal wt. only at 50 mg/kg-day ($p < 0.05$; either control group). "Some" treated from all groups had "spongy, soft"

Reference	Animal Model, N/group	Exposure Details, Timing of Evaluation	Test Agent, Doses or Concentrations	Outcomes Assessed	Maternal Toxicity	Developmental Toxicity
						spinal cords, or a rudimentary 14th rib.
Mantilla-Plata et al., (1975)	Mice, Swiss Webster N = 5-11/treated group, 14-34 controls	Injection, i.p, daily on GD 6-15, or GD 8-10, or GD 12-14; or GD 8-9, 10-11, or 12-13; or one of GDs 8, 10, 12, 14, or 16 Evaluation on GD 19	Δ^9THC (97% pure) in 10% Tween 80 and saline. Doses: 0, 40, 80, 100 mg/kg-day on GD 6-15; 0, 25, 50, 75 mg/kg-day on GD 8-10 or 12-14; single dose of 0 or 300 mg/kg on one of GDs 8, 10, 12, 14, or 16; 0 or 50 mg/kg-day on GD 8-9, 10-11, or 12-13. 2 control groups: untreated or vehicle.	Maternal bw recorded on days of treatment and at sacrifice. Standard litter data, fetal examinations for external, internal, and skeletal anomalies.	Not noted.	↑ resorptions: At all doses ($p < 0.05$) with exposure GD 6-15. At 25, 50 mg/kg-day ($p < 0.05$) with exposure on GD 8-10. At 75 mg/kg-day with exposure on GD 12-14. At 300 mg/kg with exposure on one of days 8, 10, 12, 14, or 16. ↓ fetal bw (per group, not litter): At 50 mg/kg-day on GD 8-9, 10-11, 12-13. At 300 mg/kg on GD 10, 12, 14, 16. (all at $p < 0.05$). ↑ cleft palate (per group, not litter) with 300 mg/kg on GD 12 or GD 14. ($p < 0.05$).
Borgen et al., (1973)	Rats, Wistar N = 6 treated, 7 control dams	Injection, s.c. Daily on GD 10-12 Eval at birth; litters culled to 8 pups on PND 0, half the pups from each group cross-fostered to	Synthetic Δ^9THC suspended with polyvinylpyrrolidone (PVP) in physiological saline. Dose: 0, 10 mg/kg Control: vehicle only	Pups evaluated at birth for viability, gross malform-ations, total sex ratio/ group, total mean birth wt./group, mean litter size. Selected male pups weighed daily, and followed	Not mentioned.	No adverse effects noted at birth. Delay in age at incisor eruption ($p < 0.05$). ↓ pup wt. on PND 4, 7, 10, 13, 17, and 21

Reference	Animal Model, N/group	Exposure Details, Timing of Evaluation	Test Agent, Doses or Concentrations	Outcomes Assessed	Maternal Toxicity	Developmental Toxicity
		dams of the other group. Male pups were "arbitrarily selected" for continued assessment.		postnatally for appearance of upper incisors, eye opening, and pinna unfolding.		
Uyemo et al., (1973)	Rats, Wistar N = 8-11/group	Injection, s.c., daily on each of GD 10-12 Evaluation: Within 10 hr of birth, then cross-fostered and followed.	Δ^9THC (93% pure) in dehydrated alcohol. Doses: 0, 30, 60, 120 mg/kg-day. Vehicle control.	Newborn pups counted, weighed, sexed, and examined for external abnormalities. Cross-fostered pups weighed on PND 8, 15, 22, 29, and 42.	Not noted	No significant somatic findings reported for any of treatment group. Birth weights and growth curves for treated animals were said to be reduced, but no data presented.
Harbison et al., (1972)	Mice, Swiss-Webster N = 6 - 8	Injection, i.p. daily on each of GD 8 & 9, 10 & 11, or 12 & 13. Eval: GD 19.	Δ^9THC in 10% Tween 80 and saline. Dose: 0, 200 mg/kg Control: vehicle only.	Numbers and positions of live, dead, and resorbed fetuses. Fetal wt. Data do not appear to have been analyzed on a per litter basis	Not mentioned	↑ resorption incidence following treatment on GD 8 & 9, or 10 & 11 ($p < 0.05$). ↓ fetal wt. with treatment on GD 8 & 9, 10 & 11, or 12 & 13 ($p < 0.05$). .

Reference	Animal Model, N/group	Exposure Details, Timing of Evaluation	Test Agent, Doses or Concentrations	Outcomes Assessed	Maternal Toxicity	Developmental Toxicity
Borgen et al., (1971)	Rats, Long-Evans N = 10/group (initially)	Injection, s.c. Daily on each of GD 1-20 Eval: at birth (experiments 1 & 2), or GD 21 (experiment 3) Cross-fostering between treated and control litters is mentioned only in the study results. No details of the study protocol are provided, nor is it clear if a separate experiment was involved.	Synthetic Δ^9THC (95% pure) in olive oil. Concurrent controls for each experiment given oil vehicle. Experiment 1 0, 0.01, 0.1, 1.0, 10 mg/kg-day. Experiment 2 0, 25, 50, 75, 100 mg/kg-day. Experiment 3 0, 50, 100, 200 mg/kg-day.	Experiments 1 & 2: Pregnancy rate, gestation length, live and total pups born, litter size at birth, pup wt. at birth, and external abnormalities. Litter size and pup wt. at weaning. Experiment 3: dams GD 21 necropsied for organ wts.. (analyzed relative to bw)	Experiment 3: ↓ bw with 50 ($p < 0.05$), 100 or 200 ($p < 0.01$) mg/kg-day. ↑ heart wt. at 200 mg/kg-day ($p < 0.05$). ↓ liver wt. at 50 ($p < 0.05$), 100 or 200 ($p < 0.01$) mg/kg-day. ↑ adrenal wt. at 50 and 100 ($p < 0.05$), or 200 ($p < 0.01$). ↑ thyroid wt. at 100 ($p < 0.05$) or 200 ($p < 0.01$). Adverse lactational affects reported for maternal gestational exposure to Δ^9THC on lactation but no data or details were provided.	Experiments 1 & 2: ↑ frequency of prolonged gestation at 10 ($p < 0.01$), 75 ($p < 0.05$), and 100 ($p< 0.01$) mg/kg-day ↓ litter size at birth with 100 mg/kg-day ($p < 0.05$) ↓ litter size at weaning with 50, 75, or 100 mg/kg-day ($p < 0.01$). Experiment 3: ↓ litter size at term with 100 ($p < 0.05$) or 200 ($p < 0.01$) mg/kg-day.

Appendix 4. Animal Neurodevelopmental Toxicity Studies

Appendix Table 4.1. Inhalation Neurodevelopmental Animal Studies

Reference	Animal Model N/group	Exposure Details and Terminal Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
Charlebois & Fried, 1980	Rats, Wistar N = 9/group 6h post-birth, litters culled to 4 females and 4 males each	Exposed dams daily from 20 days premating through gestation day (GD) 20. Chamber exposures for 9 min in closed box, with 30 sec ventilations halfway through exposure period. Evaluation at birth and different postnatal days (PND), depending on the test	Cannabis smoke (CS) group: 0.6 g cannabis, containing 1% Δ^9THC, in filter-tipped cigarette tubes. 2 control groups: placebo smoke (PS) from cannabis plant material with cannabinoids removed, or no smoke (NS). For each type of exposure (CS, PS, or NS), there were three different groups each receiving a diet, containing a speciried protein level: 8, 24 or 64% protein.	<u>Locomotor activity</u> on PND 1, 7, 14 and 21 and in an <u>Open-field</u> situation on PND 30, 31, and 32, with each trial being over a 3-min period separated by 24 hr. 2 familiarization trials (quantifying ambulation and rearing behaviors) and 1 exploratory trial (ambulation, rearing and time spent near the novel object) <u>Water Maze</u> began on PND 35. On each trial: time taken to reach the 1st choice point, the number of errors, and the total time in the water. Also tested after a 48-hr retention interval on the same variables (Maternal wt. gain measured on GD 5, 10, 15, and 19.)	↑ gestation length for CS group on 8% protein diet ($p < 0.002$). ↓ wt. gain GD 15-18 for all 8% protein groups ($p < 0.5$).	All comparisons to NS and PS controls on corresponding % protein diet. <u>Familiarization trials</u> Delayed righting reflex observed for CS group on 8% protein diet (p value not provided). <u>Locomotor activity</u> ↓ activity on PND 21 with CS combined with all diets. <u>Open field</u> ↓ Ambulation score for 8-CS on PND 31 and 32

Reference	Animal Model N/group	Exposure Details and Terminal Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
Fried and Charlebois, 1979	Rats, Wistar Experiment I: gestation N = 4/group Experiment II: pre-concept-ion, female N = 6/group Experiment III: pre-concept-ion, male N = 8/group Experiment IV: pre-conception and gestation, female N = 6/group (initially) Litters culled to 8/dam PND 0	Each experiment: Whole body exposure for 19 min/day. Exposure periods: Experiment I: GD 1 – 19 Experiment II: 19 days prior to mating Experiment III: 19 days prior to mating Experiment IV: 19 days prior to mating and through gestation	All 4 experiments: 0.6 g cannabis containing 1.1% Δ^9THC, estimated to provide 3.3 mg/day (or 16 mg/kg-day) Δ^9THC exposure. Controls exposed to smoke from cannabis plant material with cannabinoids removed	All 4 experiments: Activity counts taken PND 7 and 14 Pups observed for day of reflex acquisition: righting reflex, free fall righting, cliff avoidance, visual placing. (Daily maternal weights; gestation length.)	None reported in any 4 experiments.	Experiment I: ↓Activity in exposed offspring compared to controls (F=9.32; df= 1/12 and p= 0.010). ↑ time to acquisition of visual placing reflex ($p < 0.001$) Findings of experiment I not observed in experiments II, III, or IV.

Reference	Animal Model N/group	Exposure Details and Terminal Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
Fried, 1976	Rats, Wistar N = 4 controls; 5 treated Pregnant rats paired by plug date, and members of pair randomly assigned to treated or control groups. 4 control and 3 treated dams delivered litters	Whole-body exposure, 9 min/day, GD 1 – 19 Initial evaluation PND 0 Culling and cross-fostering of half-litters from controls (C) and exposed (E) groups: C-C, C-E, E-C, and E-E Multiple postnatal evaluations through PND 14	0.6 g cannabis containing 1.1% Δ^9THC, estimated to provide 3.3 mg/day (or 16 mg/kg-day) Δ^9THC exposure Controls exposed to smoke from cannabis plant material with cannabinoids removed.	Activity evaluated on PND 1, 7 and 14. Timing of acquisition of behavioral reflexes: righting, free fall righting, cliff avoidance, visual placing No maternal outcomes Non-neurodevelopmental outcomes are described in Appendix 2 of this document.	Not mentioned	PND 7: ↓ activity in E-C and E-E offspring compared to C-C and C-E controls ($p < 0.02$).

Neurodevelopmental Toxicity Studies in Animals Exposed to Δ⁹-THC or a Cannabis Extract via the Oral Route

The studies are organized in three tables, according to when exposures occurred:

1) pre-conceptual exposures to parents only,
2) maternal exposure during pregnancy resulting in in utero exposure of offspring only, or
3) perinatal exposure in which the dam was exposed during both the prenatal and postnatal period and exposure to the offspring occurred via lactation as well as in utero.

Within each of the tables, the studies are organized by date of publication, from most recent to oldest. Most studies in animals on the neurodevelopmental toxicity of Δ⁹-THC or a Cannabis Extract via the Oral Route have been included in the table, however; some studies are only discussed in the main text of this document because they were less informative than the tabulated studies.

Appendix Table 4.2. Oral Neurodevelopmental Animal Studies: Pre-Conceptual Exposure

Reference	Animal Model N/group	Exposure Details and Terminal Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
Levin et al., (2019)	Rats, Sprague-Dawley N=6 Controls * N=7 Treated * *N in article's Results section not consistent with N in Methods, i.e., N=9 Control; N=8 Treated 1 male and 1 female offspring from each litter were tested Litter was the unit of variance	Paternal exposure. Daily gavage for 12 days, ending one day before mating Controls received vehicle. After exposure, males were bred with drug-naive females. Offspring were tested at the following time-points: postnatal weeks 4, 5, 6, 7, 8-11, 12-40	Δ^9-THC: 2 mg/kg-day [Sigma-Aldrich St Louis, MO, USA] Vehicle: 10% ethanol, 1% Triton X-100 in saline.	Clinical health of the offspring (litter size, sex ratio, pup birth weight, survival and growth). Tests of locomotor activity, emotional function and cognition in offspring, as follows, by postnatal week (wk): Wk 4: Elevated Plus maze Wk 5 (Adolescence) & Wk 18-20 (Adulthood): Figure 8 Locomotor Activity Wk 6: Novelty Suppressed Feeding Wk 7: Novel Object Recognition Wks 8-11: Radial Arm Maze Wks 12-40: Operant Visual Attention Task	Not applicable (paternal exposure study)	Elevated plus maze or radial-arm maze: No effect. Figure-8 maze: Small but significant ↑ in habituation of activity in adult offspring but not in adolescent offspring ($p < 0.05$). Novelty suppressed feeding test: Trend toward ↓ in the latency to begin eating ($p=0.12$). Novel object recognition test of non-spatial memory: No significant effects ($p<0.0005$). 16 arm radial maze test of spatial memory: No significant effects in error rates ($p<0.0005$). Operant attention task during adulthood: Significantly ↓ percent correct (84.1 ± 0.7) compared to controls (87.4 ± 0.7), with a ↓ in percent hit performance (THC 79.6 ± 1.0%; Control=84.9 ± 1.5%), Percent correct rejection was not significantly affected.

Appendix Table 4.3. Oral Neurodevelopmental Animal Studies: Prenatal Exposure

Reference	Animal Model N/group	Exposure Details and Terminal Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
Gomez et al., (2003)	Wistar rats For each gender, 6 fetuses from 3 different litters were collected Measurements were pooled from 2 brain sections per fetus. At least 4 animals per experimental group	Oral dose GD 5 - 21 Evaluation at gestation day (GD) 21 of the ontogeny of specific neurotransmitters in offspring of exposed dams.	Δ^9-THC: 5 mg/kg [GW Pharma-ceuticals Salisbury, UK] Vehicle: sesame oil	mRNA levels of neural cell adhesion molecule (N-CAM), a key protein for neural development referred to as L1, analyzed by in situ hybridization. Histochemistry in pup brains.	Not reported	L1 mRNA levels varied with brain region. Level were ↑ in different white matter areas -fimbria, stria terminalis, stria medullaris, corpus callosum - but not in fornix and fasciculus retroflexus. Levels were ↑ in grey matter structures of the septum nuclei, habenula, paraventricular thalamic nucleus, and cortical subventricular zone but not in the cerebral cortex, basolateral amygdaloid nucleus, hippocampus, some diencephalic structures, basal ganglia. The increases reached statistical significance only for males. Regions with ↑ L1 mRNA levels also exhibited an abundant expression of cannabinoid CB1 receptors. L1-mRNA was unchanged in the fornix and fasciculus retroflexus. Authors hypothesize that Δ^9-THC may ↑ glial cell L1 expression not neuronal L1.

Reference	Animal Model N/group	Exposure Details and Terminal Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
Bonnin et al., (1996)	Wistar rats (Unclear N. Authors state at least 5 or 6 determinations/group)	Route, unspecified. (Likely oral gavage due to sesame oil vehicle.) GD 5 until killed. Animals killed at GD 14, GD16, GD18, GD21, PND1 and PND 5. Fetal brains removed and immediately frozen at -70°C until assayed.	Δ^9-THC: 5 mg/kg-day [THC source: NIDA, USA] Vehicle: Sesame oil Plasma and tissue THC concentrations determined by radioimmunoassay	Gene expression and mRNA levels of Tyrosine hydroxylase (TH) and protein levels and catecholamine levels in fetal brain	No changes in maternal water and food intake, maternal weight gain and litter size	↑TH mRNA levels and protein levels and catecholamine levels in fetal brains at GD14; levels normalized at GD16. ↑TH mRNA levels in females but not in males exposed from GD 18 - GD 21 with sometimes parallel effects on protein levels.
Bonnin et al., (1995)	Rats, Wistar (Unclear N. Authors state at least 10 determinations/ group)	Route, unspecified. (Likely oral gavage due to sesame oil vehicle.) Daily from GD 5 through evaluation on GD 14 or GD 16 Fetal brains removed and immediately frozen at -70°C until assayed	Δ^9-THC: 5 mg/kg-day [NIDA, USA] Vehicle: Sesame oil Plasma and tissue Δ9-THC concentrations were determined by radioimmunoassay	At GD 14 and 16 Thyroid hormone (TH)-mRNA concentrations measured by Northern blot analysis with a specific TH probe in the brain (Values are means of at least 4 different blots). Analyses of the activity of TH and the contents of catecholamines [DA and norepinephrine (NE)] performed by HPLC	None reported	↑ TH gene expression (2-fold) and TH enzyme activity (3-fold) in brain catecholaminergic neurons in fetuses at GD 14. Catecholamine contents not detectable. Both TH gene expression and TH enzyme activity normalized at GD 16. $P < 0.05$
Hutchings et al., (1991a)	Wistar rats N=8 in each Δ^9-THC treatment group Controls: Nontreated (NT) N= 9 Pair-fed (PF) N=7 Litter used as the unit of analysis for weight and startle response	Oral route (via gastric intubation) GD 2- GD 22	Δ^9-THC 15 or 30 mg/kg-day [From NIDA, USA] Vehicle: sesame oil	Startle response test 2 separate analyses of startle response Amplitude performed on the 121 stimulus presentations: First presentation and repeated measures over 5 blocks of 24 trials.	↓ In food intake of dams at 30 mg/kg-d, $p < 0.001$ ↓ Weight gain in dams at 15 and 30 mg/kg-d and PF dams $p<0.001$	No effects on startle response amplitude seen in either dose group $P<0.05$ (Offspring mortality for THC groups > controls but not significant.)

Reference	Animal Model N/group	Exposure Details and Terminal Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
Abel et al., (1990a)	Rats, Long Evans Pregnant dams of different ages: 3 mo (N= 53) 4 mo (N= 38) 6 mo (N= 37)	Oral, "Intubated" (presumed gavage) Daily from GD 6 to parturition. Behavior tests at PND 16 and 17	THC (not specified if Δ^9 form), From NIDA, USA 0, 10 or 25 mg/kg-day Vehicle: sesame oil Animals in the 0 and 10 mg/kg groups were pair fed to receive the same food intake as those given the 25 mg/kg dose	Spontaneous Alternation PND 16 a) Trial latency b) Trials to criterion (n=11-20 per sex per group) Passive Avoidance PND 17 (n= 11-20/sex/group)	↓ Maternal weight gain $p < 0.001$ Did not affect litter size	No significant effects in either spontaneous alternation or passive avoidance tasks
Abel et al., (1990b)	Long Evans Hooded Rats N= 10/group 2 males or 2 females from each group were evaluated	Presumed oral route (Intubated) GD 6 to parturition.	<u>Study 1:</u> 50 mg/kg of Δ^9-THC or Δ^9-THC plus alcohol (1g/kg). <u>Study 2:</u> Δ^9-THC (0, 25 or 50 mg/kg) or THC (0, 25 or 50 mg/kg) plus alcohol (0, 1 or 2 g/kg) Vehicle: sesame oil Animals were intubated with Δ^9-THC and three hours later with alcohol Animals in each of the Δ^9-THC subgroups were pair-fed to the subgroup receiving the highest alcohol dose	PND 17 Passive Avoidance Test Blood Δ^9-THC and metabolite levels	↓ maternal weight gain during pregnancy at 25 mg/kg-d Δ^9-THC, $p < 0.001$	No significant effects seen in passive avoidance test

Reference	Animal Model N/group	Exposure Details and Terminal Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
Brake et al., (1987)	Wistar rats N= 10 for THC groups N= 9 for pair fed (PF) or non-PF vehicle control groups for nipple attachment N= 8 litters/group for locomotor activity tests (The PF vehicle control group was only allowed to consume the same amount of food and drink as consumed by the high dose THC group.)	Gastric intubation GD 8-GD 22. Tested at 3-day intervals from PND 2 to 32 for differences in locomotor activity. Tested for nipple attachment on PND 2, 5, 8, 11, and 14 Litters were culled to 10 pups; and those containing < 8 pups were sexed and weighed but excluded from behavioral testing.	15 or 50 mg/kg-d Δ^9-THC (source not specified) Vehicle: Sesame oil "All treated and control litters were fostered at birth to untreated dams."	Locomotor Activity Latency for Nipple Attachment	↓ of food and water intake by treated dams, no data provided	No effects seen in either dose groups for locomotor activity. The 50 mg/kg-d and PF litters took longer to attach on Days 5-8 than the 15 mg/kg-d and non-PF litters $p < 0.05$ The authors concluded the nipple attachment effect may not be an effect of Δ^9-THC, but due to a decrease of food and water intake of dams.
Dalterio et al., (1984)	Mice (strain not specified) Unclear how many dams/group. For plasma T, LH and FSH levels N= 5 pups (vehicle) N= 3 pups (THC) For castrated adults: N= 9-12 (THC) N= 14-15 (vehicle) 20 µg T challenge	Oral route. On GD18 and on one of the last four days of gestation.	Δ^9-THC 50 mg/kg-d [source not specified] Vehicle: sesame oil	Plasma T, LH, and FSH Levels Biogenic Amine Concentrations Testicular Responsiveness to Gonadotropins Organ Weights and Testicular T Levels *In vitro* T Production	None reported	In Δ^9-THC exposed: ↓ Plasma T and FSH levels, $p < 0.05$ in castrated males challenged with T ↑ Responsivity of testis to in vivo intratesticular LH administration $p < 0.05$ ↑ Weight of testes in Δ^9-THC exposed animals $p < 0.05$ No change in plasma hormone levels No significant effect on brain biogenic amines concentrations (The study reported on other cannabinoids. Only results of Δ^9-THC exposure reported here.)

Reference	Animal Model N/group	Exposure Details and Terminal Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
Abel et al., (1984)	Long-Evans hooded rats N= 10-14/group for evaluation of spontaneous alternation N= 10/group for evaluation of two-way shock avoidance N= 6/group for rotorod behavior test	Presumed oral via intubation since oil vehicle control was used GD 1, 2, 3, 4 and 5 The 50 mg/kg-day group continued on GD5 – GD21 Behavioral tests at PND 36, 48 and 77	Δ^9-THC: 5, 10, 20,30,40 or 50 mg/kg-day [From NIDA, USA] Vehicle: olive oil Group (G50): 50 mg/kg-day Group (G150): 150 mg/kg-day Group (G0) pair-fed vehicle Group C: Non treated ad lib control	Spontaneous Alternation: Females tested at PND 36 Two-way shock avoidance: Females tested at PND 48 Rotarod Behavior: Females tested at PND 77	↓ # of pregnancies carried to term in a dose-related manner $p < 0.001$ ↓ Maternal weight gain both absolute and net ($p < 0.001$) for both doses compared to both control groups No effects on gestation length or litter size	No effects on spontaneous alternation, shock avoidance, or rotarod behavior in G50 group. No viable litters in G150 group, so neurobehavioral testing of offspring could not be performed.

Reference	Animal Model N/group	Exposure Details and Terminal Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
Vardaris et al., (1976)	Sprague-Dawley Rats N= 8 Δ^9-THC N= 8 Vehicle N= 5 litters/group for behavior, remaining pups used for biochemical analysis.	Oral route Once a day GD 3 – parturition Behavior was examined on PND 20, 21 and 90	Δ^9-THC: 2 mg/kg-day [From NIDA, USA] Vehicle: aqueous suspension in the presence of polyvinylpyrrolidone (PVP)	Pup tissue levels of Δ^9-THC and metabolites Spontaneous Social Behavior Push Tube Test Passive Avoidance Task (5 trials)	↓ Weight in Δ^9-THC exposed dams (not significant) 1 abnormal pregnancy in Δ^9-THC treated group with litter containing 3 stillborn pups; 7 healthy pups	1st trial for passive avoidance latencies: longer initial latencies in Δ^9-THC treated groups $p < 0.05$ 3rd trial: Significantly lower in acquisition of passive avoidance behavior in Δ9-THC mice (p value not reported No significant change in spontaneous social behavior Shorter latencies in winning behavior in Δ^9-THC treated mice in push tube test, but not significant "Rats whose dams had received the drug forced control animals to back out of a push tube in 67% of the tests at 21 days of age and 94% of the tests at 90 days of age."

Appendix Table 4.4. Oral Neurodevelopmental Animal Studies: Perinatal Exposure

Reference	Animal Model N/group	Exposure Details and Terminal Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
Beggiato et al., (2017)	Rats, Wistar, male offspring: N= 7-10 for spontaneous [^{3}H]-GABA outflow N= 7-8 for K^+-evoked [^{3}H]-GABA outflow N= 6 for [^{3}H] GABA uptake N= 7 for CB_1 receptor (B_{max}) binding *N: Number of animals perinatally exposed to THC or vehicle. One male pup was used for each of the GABA outflow experiments (to perform either the challenge, or the basal or the uptake).	Gavage Gestation day (GD) 15 - postnatal day (PND) 9. (Lactational exposure) Male offspring examined at PND 90. Pharmacological challenge with either Δ^9-THC (0.1 µM) or WIN55,212-2 (2 µM), to assess K+-evoked [^{3}H]-GABA outflow	Δ^9-THC 5 mg/kg/day [Sigma, Milan, Italy] Vehicle: sesame oil	Hippocampal slices from euthanized 90-day-old rats assessed for spontaneous and Δ^9-THC challenged [^{3}H]-GABA outflow, CB_1 receptor (B_{max}) binding	No significant effects on dam weight gain during gestation or lactation, or on pregnancy length (data not shown).	Significantly ↓ basal and K^+-evoked [3H]-GABA outflow, along with a ↓ in [^{3}H] GABA uptake. Significant ↓ of B_{max} binding ($p<0.01$). No differences in K_d value. ↓ of K^+-evoked [^{3}H]-GABA outflow in response to a pharmacological challenge with Δ^9-THC (0.1 µM) significantly blocked by adding the selective CB_1 receptor antagonist SR141716A (permanently decreases hippocampal CB_1 receptor).
Castaldo et al. (2010)	Rats, Wistar Hippocampal slices N=13/group (Glutamate outflow experiments) N=12/group (GLT1 and GLAST expression and glutamate uptake experiments) One male pup/litter was used for each experiment	Buccopharyngeal cannula GD 15 - PND 9 (Lactational exposure) Male offspring assessed PND 40:	Δ^9-THC 5mg/kg [Sigma, Milan, Italy] Vehicle: Sesame oil To evaluate the effect of Δ^9-THC on K^+-evoked glutamate release, 0.1µM Δ^9-THC was added to the superfusion medium, with or without 100nM of the CB_1 antagonist	Using HPLC, hippocampal slices from euthanized 40-day-old rats assessed for K^+-evoked glutamate release and L-[^{3}H]-glutamate uptake. Protein expression of glutamate transporters (GluTs) in hippocampal synaptosomes (by western blot).	No significant difference in litter size, maternal food intake, or weight gain.	L-[^{3}H]-glutamate uptake was significantly ↓ in hippocampal slices from Δ^9-THC-rats. Loss of the stimulatory effect of Δ^9-THC (0.1µM) on hippocampal glutamate release in Δ^9-THC-rats. Significant ↓ in glutamate transporter 1 (GLT1) and glutamate/aspartate transporter (GLAST) protein in hippocampal synaptosomes compared to controls.

Reference	Animal Model N/group	Exposure Details and Terminal Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
Trezza et al, (2008)	Rats, Wistar Litters culled to 8 pups/litter 1 male/litter used per test N=12/group for treated groups N=20–26 for vehicle control	Buccopharyngeal cannula GD 15 - PND 9 (Lactational exposure) Offspring assessed at PND 12, 35, 80	Δ^9-THC: 2.5, 5 mg/kg [Sigma, Milan, Italy] Vehicle: Sesame oil	Emotional reactivity of offspring investigated using isolation-induced ultrasonic vocalization in infant, social interaction in adolescent, and elevated plus-maze tests in adult offspring	Perinatal THC treatment did not affect reproduction parameters of dams	At 5 mg/kg: Rat pups (PND 12): ↑ number of ultrasound vocalizations ($p<0.05$), not accompanied by any changes in locomotor activity. Adolescent rats (PND 35): inhibited social interaction and play behavior. Adult offspring (PND 80): anxiogenic-like profile in elevated plus-maze test with ↓ time spent on the open arms.

Reference	Animal Model N/group	Exposure Details and Terminal Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
Campolongo et al., (2007)	Rats, Wistar, Male offspring: N= 18/group for microarray analysis N= 10/group for *in vivo* microdialysis Behavioral studies: Inhibitory Avoidance N= 14 (vehicle) N=14 (THC) Social Discrimination N=12 (vehicle) N= 11 (THC)	Buccopharyngeal cannula GD 15 - PND 9. (Lactational exposure) Male offspring assessed PND 80 (Inhibitory Avoidance and Social Discrimination test only)	Δ^9-THC 5 mg/kg [Sigma, Milan, Italy] Vehicle: sesame oil Controls received the same volume of vehicle (5 mg/ml) as treated animals.	*Microarray analysis* to identify global gene expression changes in the prefrontal cortex. *In vivo microdialysis* for glutamate and norepinephrine *Behavioral studies*: Inhibitory avoidance: Time taken to completely enter into the dark compartment (an index of non-associative behavior). Latency to reenter dark compartment after a shock (24 hours prior) (as a measure of memory retention). For social discrimination: a 5-minute learning trial and 5-minute retrieval trial, separated by 30-minutes. Juvenile recognition determined by the time spent by the adult during the 2nd exposure with the previously exposed juvenile (3-4 wk old male) as compared to the novel juvenile.	No differences were observed in body weight gains of Δ^9-THC-treated dams during gestation or lactation and no effect on pregnancy length or litter size at birth.	*Microarray analysis* Perinatal Δ^9-THC ↓ genes related to myelination; ↑ genes involved in apoptosis. *In vivo microdialysis* Basal extracellular cortical levels of glutamate and norepinephrine were significantly ↓ in perinatal Δ^9-THC group. *Behavioral studies* Inhibitory avoidance test: No difference observed during the acquisition trial, but perinatal Δ^9-THC group had a ↓ ability to remember the task when the trial was repeated 24 h later ($p<0.01$). Social discrimination test: Perinatal Δ^9-THC affected discrimination abilities at adulthood during the retrieval trial. Controls investigated the novel juvenile significantly longer than the familiar rat ($p<0.01$).

Reference	Animal Model N/group	Exposure Details and Terminal Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
Economidou et al., (2007)	Rat, Wistar N= 8-9/group. Groups: - Δ^9-THC - Ethanol - Ethanol + Δ^9-THC - Vehicle One rat per litter per treatment group.	Gavage GD 15 - PND 9 (Lactational exposure) Male offspring assessed at PND 80 for microarray analysis	Δ^9-THC: 5 mg/kg [Sigma-Aldrich, St. Louis, MO, USA] Vehicle: Sesame oil	Ethanol self-administration and alcohol-seeking behavior in adult offspring Changes in brain gene expression profiles in adult offspring by microarray	No effect on dam body weight or litter size (Litter as unit)	No effect on ethanol self-administration in adults in any treatment group. 139, 112 and 170 genes were differentially expressed in the ethanol, Δ^9-THC, and ethanol + Δ^9-THC groups, respectively,. $P<0.05$.
Moreno et al., (2005)	Rats, Wistar 4 litters/group N=17–24/sex/group	Oral (not otherwise specified) GD 5 - PND 24 (Lactational exposure) Offspring evaluated at PND 70.	Δ^9-THC (>95% purity) at 0.1, 0.5 or 2 mg/kg-day [NIDA, USA] Vehicle: Sesame oil (in a volume of 0.1 ml)	Plasma corticosterone levels Behavioral outcomes: Immobility- time spent by the animals in absolute quietness (in seconds) Exploration - the sum of rearing and sniffing activities Grooming -time spent by the animals in self-cleaning. Locomotion - registered by the photocell beam system in the cage (measured as number of crossings during 5 min scoring periods over a 125 min session).	No significant changes noted in gestational and lactational parameters such as mother's food and water intake, weight gain, length of gestation, litter size and weight	Alterations in plasma corticosterone levels (↓ in males and ↑ in females $p<0.05$). Immobility - ↑ time by adult males at 120 min at all doses and for females at 60 and 120 min at low and mid- dose. Exploration - ↓ exploratory behavior only in females at low doses (0.1and 0.5 mg/kg-day). Grooming – No significant changes in any group or sex. Locomotion – ↓ activity in males at 120 min for 0.1 and 2 mg/kg and in females at 0.1 and 0.5 mg/kg at 5, 60 and 120 min. Lack of habituation in females at 2 mg/kg. Authors hypothesize that behavioral alterations were related to effects on the HPA axis.

Reference	Animal Model N/group	Exposure Details and Terminal Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
Suarez et al., (2004)	Rats (strain not specified) 6 male and 6 female offspring evaluated	Daily oral dose from GD 5 - PND 20 (Lactational exposure) Offspring evaluated on PND 20, 30 and 70	Δ^9-THC: 5 mg/kg (Source not available) Vehicle: Sesame oil	Immunohistochemical analysis of the expression of specific glutamate transporter subtypes (GLAST and EAAC1) in cerebella.	Not reported	↓Expression of the glutamate transporter GLAST in astroglial cells and EAAC1 in Purkinje neurons in exposed offspring at all ages, but mainly in males. GLAST levels lower in males and females Optical density values of EAAC1 ($p<0.05$) ↓ at PND 20 and PND 30. Authors postulate that down-regulation of the expression of glutamate transporters could be related to impairment of glutamatergic innervation of Purkinje cells.

Reference	Animal Model N/group	Exposure Details and Terminal Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
Gonzalez et al., (2003)	Rats, Wistar (Unclear N. Authors state at least 6 animals/ group)	Route, unspecified. (Likely oral gavage due to sesame oil vehicle.) Daily from GD 5 through PND 24 After at least 10 weeks after birth, offspring by gender divided in 3 series (i) 1/3 sacrificed immediately before operant morphine self-administration (ii) 1/3 for operant morphine self-administration and sacrificed immediately after last testing day (iii)1/3 operant morphine self-administration, but, after the last testing day, animals changed to saline to extinguish the morphine response and, 15 days later, sacrificed. All groups - brains removed and rapidly frozen. Using separate groups of animals examined by operant food-reinforced behavior.	Δ^9-THC: 5 mg/kg-day [NIDA, USA] Vehicle: Sesame oil	1. Acquisition of morphine self-administration behavior under PR schedule of reinforcement 2. Operant food-reinforced behavior 3. DA and DOPAC determinations	Not reported	*Morphine self-administration behavior* Female offspring showed ↑ response rate than male offspring for acquisition period and was equal for Δ^9-THC and vehicle offspring *Food-reinforced behavior* Significant differences between genders, $P < .0005$ for food-reinforced behavior, not affected by Δ^9-THC *Neurochemical determinations* DOPAC/DA ratio - effect of perinatal Δ^9-THC [nucleus accumbens and ventral tegmental area, $P < .05$], but interacting with gender before morphine self-administration DOPAC/DA ratio in the nucleus accumbens and ventral tegmental area of females in Δ^9-THC was < vehicle. These changes, disappeared after daily sessions (15 days) of morphine self-administration and did not reappear after 15 additional days of extinction of this response.

Reference	Animal Model N/group	Exposure Details and Terminal Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
Moreno et al., (2003)	Rats, Wistar N=59 Vehicle control N=49 0.1 mg/kg-d N=45 0.5 mg/kg-d N=39 2 mg/kg-d	Oral NOS GD 5 - PND 24 (Lactational exposure) Assessed for behavioral endpoints > PND 70	Δ^9-THC: 0.1, 0.5, 2 mg/kg-d Vehicle: Sesame Oil Dopamine (D_2) agonists used: 0.1 mg/kg apomorphine sc 0.5 mg/kg quinpirole sc	Immobility: time spent by the animals in absolute quietness (seconds) Stereotypes: repetitive behavioral acts) Locomotion: measured as number of crossings Dopamine 2 (D_2) receptor sensitivity Behavioral responses: measured 5, 60, and 120 mins after treatment with D_2 agonists (apomorphine, quinpirole)	↑ average daily food and water intake Average of maternal weight gain (the difference between the weight in the day before delivery and the weight before mating) was highest in dams in the 2 mg/kg-d group No statistics provided	Immobility: $p < 0.05$ ↑ Males: 0.1 & 2 mg/kg-d↑ Females: 0.1 & 0.5 mg/kg-d Locomotion: $p < 0.05$ ↓ Males: 2 mg/kg-d ↓ Females:0.5 mg/kg-d ↑ immobility in males with apomorphine and potentiation of apomorphine-induced decrease in locomotion $p < 0.05$ ↑ sensitivity effect to the immobility induced by quinpirole but effects subsided (only in males) $p < 0.05$ ↓ locomotion score in females in all dose groups 5 min after quinpirole $p < 0.05$ ↑ sensitivity to presynaptic inhibition by the stimulation of D_2 autoreceptors or ↓ postsynaptic sensitivity of D_2 receptors Postsynaptic effects of the D_2 receptor agonist not enhanced.

Reference	Animal Model N/group	Exposure Details and Terminal Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
Vela et al., (1998)	Wistar rats Morphine self-administration and operant food reinforced studies n=12 for each sex n=6 for each condition μ opioid receptors autoradiographic studies: n=8 for each sex; n=4 for each condition	Daily oral dose GD5 - PND 24 (Lactational exposure)	Δ^9-THC: 5 mg/kg Vehicle: sesame oil THC from NIDA, USA	Maternal and neonatal weight gain, maternal food and water intake, gestational length, placental and fetal weights, litter size and maternal plasma Δ^9-THC concentrations. Acquisition of morphine self-administration and operant food reinforced behaviors. Opioid receptor autoradiography in brains of exposed fetuses	No nutritional deficit from THC exposure (data not reported here)	In females but not males, statistically significant ↑ in acquisition rate of intravenous morphine self-administration behavior (on the last day of the acquisition period). Accompanied by variations in μ-opioid receptor binding in brain regions related to drug reinforcement. Reinforcing value of food independent of sex and perinatal treatment.
Rubio et al., (1998)	Wistar rats n = 50 vehicle n = 46 (1 mg/kg THC) n = 40 (5 mg/kg THC) n = 36 (20 mg/kg THC) 10 animals per sex per treatment for behavioral endpoints	Single oral dose each day to dams GD 5 – PND 24 (lactational exposure)	Δ^9-THC: 1, 5 or 20 mg/kg-day From NIDA, USA Vehicle: sesame oil	Morphine-induced place preference (CPP) studies Defensive withdrawal test Plasma levels of adrenocorticotropic hormone (ACTH) and corticosterone after the end of the CPP test	No differences in maternal weight gain, or in the size and weight of the litters	↑sensitivity to the reinforcing effects of morphine in the adult male offspring, sexually dimorphic behavioral and endocrine alterations in adaptive responses to stressors (significant in male offspring at 1 mg/kg and 5 mg/kg and in female offspring at 1 mg/kg. Effects not seen at 20 mg/kg.)

Reference	Animal Model N/group	Exposure Details and Terminal Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
Navarro et al., (1996)	Wistar rats Number of animals per group not stated	Oral route GD 5-PND 24 (Lactational exposure) At PND 70 offspring assessed for behavioral and neurochemical studies.	Hashish crude extract equivalent to THC 20 mg/kg (Spanish Administration) Vehicle: Sesame oil	<u>Behavioral Studies</u> Dark-light emergence test, Socio-sexual approach behavior test Social Interaction test <u>Neurochemical studies</u> Tyrosine Hydrolase Dopamine L-3,4-dihydroxy-phenylacetic acid (DOPAC) D1 dopamine binding site analysis for each limbic forebrain area (olfactory tubercles, rostral limbic nuclei, nucleus accumbens, septal nuclei and parts of the anterior amygdaloid nuclei).	None reported	Sociosexual approach behavior test. Compared to controls, hashish-exposed males spent more time in the vicinity of the incentive female, with ↓ frequency of visits to, and time spent in, the male incentive area. This behavior was normalized and even inverted later. No changes in spontaneous locomotor activity (no data shown). A significant ↓ in DOPAC contents in the limbic forebrain ($p < 0.05$) was seen in males, suggesting a ↓activity of mesolimbic dopaminergic neurons. No effects seen in females.

Reference	Animal Model N/group	Exposure Details and Terminal Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
Rubio et al., (1995)	Wistar rats Δ^9-THC: n = 8 Control: n = 7 10 animals per sex per treatment for behavioral endpoints	Oral route GD 5 - PND 24 (lactation exposure postnatally) Evaluation on PND 79	Δ^9-THC: 5 mg/kg-day [From NIDA, USA] Vehicle: sesame oil 30 µg/kg ip morphine	Ontogeny of motor behaviors in adult male and female offspring of exposed dams. Tested for Locomotor activity Rearing Sniffing Time spent grooming Fecal boluses Morphine place preference conditioning (MPP) Neuroendocrine Parameters corticotropin releasing factor (CRF-41) in medial basal hypothalamus (MBH) Plasma levels of CRF-41 and adrenocortico-tropic hormone (ACTH)	None reported	In female offspring: ↑ Locomotor activity ↑ Rears ↑ Sniffing ↑ Time spent grooming In male offspring: ↑ Exploratory behavior in a plus-maze: ↑ Rears ↑ Sniffing ↑ Time spent grooming ↑ Fecal boluses ↑ Sensitivity to rewarding effects of morphine noted in both sexes (marked in the males). $p < 0.05$ ↑Levels of plasma corticosterone and CRF in the medial basal hypothalamus in offspring (females >male). $p < 0.05$

Reference	Animal Model N/group	Exposure Details and Terminal Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
Navarro et al, (1994)	Wistar rats Δ^9-THC: n=7 Vehicle: n=8	Oral route GD 5 – PND 24 (Lactational exposure) Evaluations at PND 15, 20, 30, 40 and 70	Δ^9-THC: 5 mg/kg From NIDA, USA Vehicle: sesame oil	Ontogeny and expression of motor behaviors. Motor behaviors exhibited in novelty-stressing conditions were evaluated in Socio-sexual approach behavior test Dark-light emergence test Plasma corticosterone levels measured using radioimmunoassay	Slight ↓maternal weight gain ($p < 0.05$) not related to a lower intake of food or water in the prenatal period and without changes in maternal weight or litter size or weight. No effect on postnatal dam weight gain or postnatal mortality. No change in litter size or weight	In Δ^9-THC animals: ↑ Rearing and locomotor activities in males and females at PND 15 and 20 (pre-weanling ages). $p < 0.05$ Effects not observed at PND 30 and 40, but were observed again in PND 70 (adult) females. $p < 0.05$ Females, but not males, exhibited ↓ locomotor activity in the socio-sexual approach test, and an ↑ in emergence latency in the dark-light emergence test. $p < 0.05$ ↓ Latency to males (males) $p < 0.05$ ↑ Time spent grooming (males and females) $p < 0.05$ ↓ External crossings (females) $p < 0.05$ THC-exposed females had an ↑ plasma corticosterone adrenal hormone, whereas males had ↓ levels. $p < 0.05$

Reference	Animal Model N/group	Exposure Details and Terminal Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
Walters & Carr (1988)	Sprague-Dawley rats N = 4 different groups of female rats dosed No other details provided	Daily oral dose began 2 weeks prior to mating through PND 20 (Lactational exposure) Evaluated at PND 10, 20, 40 or 60.	Δ^9-THC: 10mg/kg [From NIDA, USA] Vehicle: sesame oil	Offspring brains assayed for development of catecholamine receptors (measured by Bmax – binding capacity): α1-adrenergic and D2-dopaminergic receptors in cerebral cortex and striatum. Striatum assayed for TH activity	No effect on maternal weight gain during pregnancy.	In Δ^9-THC exposed: No effect on B_{max} of D_2 receptors in the striatum ↑ in B_{max} on PND 20 in α_1-adergic receptors in the cerebral cortex $p < 0.05$ No significant change in TH activity
Golub (1981)	Rhesus monkey (Macaca mulutta) Δ^9THC: N=5 Controls: N=8	Oral route (Injected into a preferred food item - cookie or prune) Δ^9-THC treated females dosed daily for 2 years prior to conception and daily throughout pregnancy and lactation (Animals weaned at 3.5 months) Behavioral alteration tested in offspring in 2 different situations at 2 different ages -1 & 2 years of age	Δ^9-THC: 2.4 mg/kg-day [From NIDA, USA] Control: Similar food item without Δ^9-THC	Test Series #1: 13 daily sessions at age 1 year. Photographs of unfamiliar scenes presented at one peephole and blank slides of equivalent light intensity were presented at the other peephole. Different pair of stimuli was used for each session Test Series #2: 20 daily sessions at age 2 years. Simple and complex images of children's toys presented at the 2 peepholes; repeated to compare response to novel and familiar stimuli	Not reported	Test Series #1: ↑visual attention in Δ^9-THC group compared to controls related to the novelty and complexity of the visual stimuli (blank or picture). $P<0.05$; $p<0.02$ Test Series #2: Δ^9-THC offspring directed ↑attention at novel stimuli compared to familiar stimuli (simple or complex) ↓ reactivity to visual stimuli

Appendix Table 4.5. Parenteral Neurodevelopmental Animal Studies

Reference	Animal Model N/group	Exposure details and Timing of Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
Vargish et al., (2017)	Mice: 5HT3AR-GFP or Nkx2.1-cre:RCE-GFP reporter lines Timed pregnancy was performed by breeding transgenic males with either wild-type C57/BL6 or transgenic females. N = 10/group (initially)	Daily intraperitoneal (i.p.) injections of pregnant mice from gestational day (GD) 10.5 – 18.5 (birth) Three mice from three litters per treatment were examined for: cholecystokinin (CCK), parvalbumin, and somatostatin Brain immunohistochemistry evaluated on postnatal days (PND) 20 - 30	Δ^9-THC: 0, 5 mg/kg (in 5% ethanol+5% corn oil in saline) [From NIAAA, NIH] Controls injected with same volume of vehicle as exposed	Interference with endogenous cannabinoid regulation of fetal nervous system development *in utero* evaluated via immunohistochemistry and cell morphology: Photomicrographs of CCK expressing interneurons (CCK-INTs). i.e., expression and location of these neurons. Density of cells labeled for CCK, parvalbumin and somatostatin	Not reported	Loss of CCK-INTs ↓Density of CCK-INTs in exposed pups compared to controls (*P =0.001). No significant difference for parvalbumin and somatostatin Strong localization of cannabinoid subtype-1 receptors (CB1Rs) to CCK-INTs but not the other cell subpopulations

Reference	Animal Model N/group	Exposure details and Timing of Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
Pitsilis et al (2017)	Sprague-Dawley rats After birth, litters were culled to 8–10 pups.	Female rats received intraperitoneal (i.p.) injections every second day during PND 28-50 (12 injections in total). Mated with drug-naïve male rats on PND 65 to 69, producing F1 pups. Assessed reward-modifying effects of Δ^9-THC and d-amphetamine on adult male offspring (F1).	Δ^9-THC [source not stated] F0: 0 (vehicle), 0.1, or 1 mg/kg. Δ^9-THC was dissolved in 5% DMSO, 5% cremophor and 90% saline), injection volume: 3 ml/kg F1: For assessing effects in F1 males of exposed F0 females, F1 males were given i.p. injections of either: Δ^9-THC at doses of 0.1, 0.5, or 1 mg/kg or d-Amphetamine at doses of 0.1, 0.5, or 1 mg/kg	Potential alteration of reward-modifying effects using the curve-shift variant of the intracranial self-stimulation (ICSS) procedure in adult male F1 offspring	No long-term, effects on behavior or other physiological parameters (e.g., gross locomotor activity, reaction to handling, social interaction, self-grooming, body weight) of the female rats, as adolescents or mothers.	The *reward-facilitating* effect of a dose of 0.1 mg/kg Δ^9-THC was abolished in F1 adult male offspring of females (F0) exposed to Δ^9-THC (0.1 or 1 mg/kg). The *reward-attenuating* effect of a dose of 1 mg/kg Δ^9-THC was not affected in F1 adult male offspring of F0 females exposed to Δ^9-THC (0.1 or 1 mg/kg). The *reward-facilitating* effect of a dose of 0.5 or 1 mg/kg d-amphetamine ↓in F1 adult male offspring of F0 females exposed to Δ^9-THC (1 mg/kg and 0.1 or 1 mg/kg, respectively). Δ^9-THC exposure of F0 females did not have long-term effects on the behavior of F1 male offspring, and did not affect lever pressing performance of F1 males (motor activity).

Reference	Animal Model N/group	Exposure details and Timing of Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
Szutorisz H et al., (2016)	Long-Evans rats 21-day old, both sexes Vehicle-exposed females were mated with vehicle-exposed males and Δ^9-THC-exposed females with Δ^9-THC-exposed males during PND 64–68 Tests for open field locomotor behavior: N=10-12/ treatment/sex For gene expression analyses: N=5/treatment/sex for adolescents N=7-8/treatment/sex for adults	F0 animals: i.p. injection, every 3rd day from PND 28-49. F1 animals: Around PND2, mixed litters of pups from Δ^9-THC and vehicle -exposed parents were cross-fostered to drug-naïve surrogate nursing mothers and weaned at ~PND24. PND35: adolescents PND62: young adults	F0 Δ^9-THC: 1.5 mg/kg From NIDA, USA Control: 0.3% Tween 80 in saline F1: untreated	Maternal weight gain, pregnancy length and fetal weights. F1: Open field locomotor behavior Gene expression analyses with quantitative reverse transcription polymerase chain reaction (qRT-PCR): striatal brain regions (dorsal and ventral striatum (nucleus accumbens)) dissected from frozen adult and adolescent brains.	Not reported	F1: ↓ locomotor movements during the first 30 minutes of open field test session ($p<0.05$) in females. No difference in males. Striatal molecular abnormalities in components of the glutamatergic system. Significant gene expression abnormalities in both male and female F1, specific to novelty reactivity in females. No general gross impairment of motor behavior in either sex.

Reference	Animal Model N/group	Exposure details and Timing of Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
de Salas-Quiroga et al., (2015)	Mice, strain not specified: Wild type (wt) CB_1R null ($CB_1^{-/-}$,Stop-CB_1) CB_1R-rescue mice: Glu-CB_1-RS (CB_1R re-expression in dorsal telencephalic neurons) GABA-CB_1-RS (CB_1R re-expression in forebrain GABAergic neurons) CB_1-RS (global CB_1R reexpression) Offspring of cross of $CB_1^{+/-}$ (male) with $CB_1^{-/-}$ (female) used for skilled motor function tests Offspring of crosses of Stop-CB_1 with Glu-CB1-RS or GABA-CB_1-RS N=4-6	F0: Daily i.p. injections of pregnant females from GD 12.5 – 16.5 (active period of glutamatergic neuron generation in the telencephalon). F1: Cortex development: subcerebral projection neurons on PND 20 Skilled motor function; seizure susceptibility on PND 60 CB_1R Western blot, receptor binding, and immunohistochemistry in GD 17.5 and PND 2.5 brain samples	Δ^9-THC [(≥99% HPLC; THC Pharm]: 3 mg/kg Control: vehicle	Developing cortex: generation of subcerebral projection neurons Corticospinal motor neuron (CSMN) - dependent motor function: skilled reaching test - the ability to retrieve a pellet of palatable food with a forelimb through a narrow slit, and staircase (skilled reaching) test Seizure susceptibility using pentylene-tetrazole (PTZ) administration paradigm. CB_1R protein levels and receptor binding in fetal and neonatal brain Immunohistological characterization of different CB_1R-rescue mice Corticospinal motor function, susceptibility to seizures in 2-month-old CB_1R-rescue mice	Maternal and neonate body weight was unaffected by Δ^9-THC treatment	F1 – Offspring exposed prenatally ↓ in number of subcerebral projection neurons in wt ↓ CB_1R protein levels and receptor binding in wt brain on GD 17.5; no effect on PND 2.5 ↓ in skilled motor function in $CB_1^{+/-}$, CB_1-RS, Glu-CB_1-RS; no effect in $CB_1^{-/-}$, GABA-CB_1-RS ↓ latency to PTZ-induced seizures in $CB_1^{+/-}$, CB_1-RS; no effect in $CB_1^{-/-}$. Partial restoration of effect (enhanced susceptibility to PTZ induced seizures by prenatal Δ^9-THC exposure) in Glu-CB_1-RS, GABA-CB_1-RS F1 – Vehicle control offspring Stop-CB_1 mice have enhanced sensitivity to PTZ induced seizures, compared to CB_1-RS mice Glu-CB_1-RS and GABA-CB_1-RS mice have intermediate sensitivity to PTZ induced seizures.

Reference	Animal Model N/group	Exposure details and Timing of Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
Szutorisz H et al., (2014)	Long-Evans rats 21-day old male and female F0: Vehicle-exposed females were mated with vehicle-exposed males and Δ^9-THC-exposed females with Δ^9-THC-exposed males during PND 64–68.	F0 animals: i.p. injection, every 3rd day from PND 28-49. F1: 4-8 animals/ group, tested at 2 time points: PND35: adolescents PND62: young adults	F0 Δ^9-THC [source not provided]: 1.5 mg/kg Control: 0.3% Tween 80 in saline F1: untreated	F1: Heroin self-administration (after catheterization on PND 56) Open-Field Locomotor Behavior (Locomotor activity, Stereotypy and time spent in the front vs back of the chamber) before and after acute (3 days) heroin deprivation. Quantitative Reverse Transcription PCR Analyses Western Blotting and NMDA Receptor-Binding Assays Electrophysiology with a protocol to induce long-term depression (LTD) in striatal slices	No complications during pregnancy and no significant group differences in parental body weight before mating, maternal gestational weight gain or pregnancy length	↓ Pregnancy rate (about 40 %) in Δ^9-THC exposed group (data not shown). F1: ↑ work effort to obtain heroin p=0.047, with no change in locomotor activity suggesting that the ↑ active lever pressing is a behavioral response specific to drug-seeking. F1 Heroin withdrawal: Increase stereotypy behavioral disturbances (p=0.02) No correlation between heroin and number of stereotyped movements. F1-THC offspring did not present an enhanced approach behavior to the front side (p=0.02) PND35: ↑ mRNA in ventral striatum PND62: ↓ mRNA at the dorsal striatum and NMDA receptor protein levels. ↓ NMDA receptor binding in the dorsal striatum. Electrophysiology:↑ LTD in the dorsal striatum, indicating altered plasticity at excitatory synapses

Reference	Animal Model N/group	Exposure details and Timing of Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
Silva et al., (2012)	Female Sprague Dawley rats N = 12 control and 9 treated. Passive avoidance: N= 82 total (two males and two females/litter) THC: 9 litters (18M: 16F) Vehicle: 12 litters Active place avoidance N= 39 total (one male and one female/litter) THC: 9 litters (9M: 8F) Vehicle: 11 litters Attention task N= 46 animals: (one male and one female/litter) THC: 9 litters Vehicle: 14 litters Amphetamine challenge N=35 total (one male and one female/litter) THC: 9 litters (9M: 7F) Vehicle: 11 litters (11M:8F)	Intravenous (i.v.) injection from GD1 - GD21 F1 Offspring - Assessed Passive avoidance on PND 22 Active place avoidance on PND 45 Attention on PND 55 Amphetamine challenge on PND 60	Δ^9-THC [NIDA]: 0.15 mg/kg Control: vehicle (pluronic acid in saline) amphetamine challenge: 1 mg/kg	3 measures of cognitive function in male and female offspring: Passive avoidance at PND 22, testing learning and long-term memory (acquisition, consolidation, extinction). 3 retention time periods - 1 hour, 1 day, 7 days Active place avoidance at PND 45, testing spatial working memory and prediction, acquisition, retention and reversal (acquisition, retention and learning flexibility) Complex attention test with distracters at PND 60.	No significant effects on maternal wt. gain, pup birth wt. or live litter size	Passive avoidance testing: F1No ↑ in latency to crossing in any of the retention time periods. Active place avoidance task: No effects on acquisition p=0.865 or retention p=0.336 but a significant treatment effect in reversal performance in males p=0.015. Attention task: No significant effect but subtle differences - treated rats performing more poorly with Δ^9-THC-pretreated animals tending to take more trials to complete the task resulting in attention deficits and impaired performance for the attention task. p=0.039 Amphetamine challenge: Treated males and females had a dampened locomotor response, p<0.001

Reference	Animal Model N/group	Exposure details and Timing of Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
DiNieri et al, (2011)	Long Evans rats Observations on male F1 only Brain tissue samples: N= 5–16 rats/group Epigenetic regulation of dopamine receptor D2 (DRD2) N=5-6 samples/group (bilateral pooled from two rats in each sample) Conditioning for morphine N=5-6 rats/group	i.v. injections daily to dams GD5 - PND 2. On PND 2, litters were culled to 8–10, and pups fostered (all pups raised by vehicle-exposed dams) Brain samples for gene expression were obtained on PND 2 and PND 62	Δ^9-THC: 0.15 mg/kg-day [From NIDA, USA] Control: vehicle (0.3 % Tween 80 in sterile saline solution)	Examined the striatal DRD2 mRNA expression on PND2 and 65 Examined the epigenetic regulation of the nucleus accumbens (NAc)*Drd2* gene in their offspring at PND65 with antibodies specific for dimethylated lysine 9 (2meH3K9) and trimethylated lysine 4 (3meH3K4) on histone H3 Conditioning for morphine behavior in the place condition task		F1 – Offspring of treated dams ↓DRD2 mRNA levels in NAc on PND 2 and PND 62 but not in the dorsal striatum ↑ 2meH3K9 repressive mark and ↓ 3meH3K4 and RNA polymerase II at the *Drd2* gene locus. ↓ D_2R binding sites in the NAc (p=0.05) but not in the dorsal striatum Preference to the dark environment ↑ place preference for the initial non-preferred compartment after low-dose morphine
Newsom & Kelly (2008)	Long-Evans hooded rats N=8/group	Subcutaneous (s.c) injection twice daily from GD1 – 22 (to the dam) and from PND 2 -10 (to the pups). Tested on PND 90	Δ^9-THC: 2 mg/kg/ injection [From NIDA, USA] Vehicle control: ethanol, Tween 80, and 0.9% saline, in a ratio of 1:1:18 Untreated control	Weight gain in dams from GD 1 to GD 22 and offspring from PND 2 to PND 10 and on PND 21, 30, 60, and 90. Battery of tests in offspring: open field activity, active social interaction, and the forced-swim test.	No significant differences in weight gained by dams.	No significant treatment –related differences in weight gained by offspring through PND 90. ↓time in the inner part of the open field and ↑ in investigation time in the test of social interaction. No differences in the forced-swim test.

Reference	Animal Model N/group	Exposure details and Timing of Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
Spano et al., (2007)	Long Evans rats Acquisition of heroin self-administration N=5-6 animals/group. Locomotor activity N=4 animals/group mRNA expression levels N= 5 animals/group	i.v. injection GD 5-PND 2 Evaluations started on PND 62	Δ^9-THC: 0.15 mg/kg [From Sigma-Aldrich, Apoteket AB, Sweden] Vehicle: 0.3% Tween 80 and diluted with 0.9% NaCl	Effects on male offspring for heroin self-administration behavior and opioid neural systems were assessed starting on PND 62 for 19 days. Brain preproenkephalin (PENK) and preprodynorphin mRNA expression levels were assessed by in situ hybridization histochemistry (ISHH) at PND 2 and 62.(adult)	No difference in weight gain during gestation or gestational length for dams	↓latency to the first active lever press ↑ heroin-seeking during mild stress and drug extinction. ↓PENK mRNA expression in the NAc on PND2, but ↑ in adulthood; No striatal changes on preprodynorphin mRNA or PENK in caudate-putamen in adults. ↑PENK mRNA in NAc and the amygdala in adults ↓heroin-induced locomotor activity and NAc opioid receptor coupling. (P<0.05)
Singh et al., (2006)	Neonatal Male Wistar rats Effects of Δ^9-THC Pre-exposure N=104 total (52/group) <u>Experiment 1:</u> Heroin Place Conditioning N= 12/group; n=72 total <u>Experiment 2:</u> Heroin-induced Fos Immuno-histochemistry (IR) N=8/group for Fos-IR; n=32 total	i.p. injection PND 4- 14. This PND period is a critical period of rat neuronal development (majority of synapses are formed) and authors state it corresponds to the third trimester of gestation in humans. At 8 weeks of age: For Heroin-Induced Place Conditioning Heroin was given s.c. at either 0.5 or 2.0 mg/ kg Fos-IR experiment: a s.c. injection of 0.5 mg/kg of Heroin	Δ^9-THC: 5 mg/kg [Australian Government Analytical Laboratories] <u>Experiment 1</u> Vehicle-Vehicle Vehicle-0.5 (H) Vehicle-2.0 (H) Δ^9-THC-Vehicle Δ^9-THC-0.5 (H) Δ^9-THC-2.0 (H) <u>Experiment 2</u> VEH-VEH Δ^9-THC-VEH VEH-(H) Δ^9-THC-(H)	Place conditioning test (under low light -animal placement in cage divided in two sections connected by a tunnel) IR in brain: caudate putamen, NAc,, lateral septum, islands of Calleja-major, bed nucleus of the stria terminalis, central nucleus of amygdala, dorsolateral and dorsomedial periaqueductal gray, ventral tegmental area, Edinger–Westphal nucleus.	Not applicable	Δ^9-THC: ↑ rewarding properties of both doses of heroin ↑heroin-induced Fos-IR in the dorsomedial caudate putamen and ↓ heroin-induced Fos-IR in the NAc (shell), bed nucleus of the stria terminalis, central nucleus of the amygdala, dorsolateral and lateral periaqueductal gray, ventral tegmental area, and Edinger–Westphal nucleus.

Reference	Animal Model N/group	Exposure details and Timing of Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
O'Shea and Mallet (2005)	Male Wistar rat pups 6 groups of 6 males and 2 females each. N= 48 Each group allocated randomly to one of the postpartum dams. On PND 21 pups were weaned and female pups were culled. 12 male rats were used for behavioral testing Δ^9-THC: N= 5 Vehicle: N= 7	s.c. injection PND 4-14: this period corresponds to the third trimester of gestation in humans (major period of synaptogenesis) Rats were tested during adulthood for 30 trials/day, 5 days/week for 5 weeks beginning on PND56.	Δ^9-THC: 5 mg/kg [From Australian Government Lab, AGAL] Control: vehicle: (15 ml Twen-80 per ml saline)	Beginning on PND 56 using a two-component food-motivated double Y-maze test for distinct spatial discrimination and delayed alternation (simultaneous assessment of reference memory and working memory).	Not applicable	No significant differences in the spatial discrimination task ↑Delayed alternation task (mean ±SEM was 24.60 ±0.40 for Δ^9-THC and 17.57 ±0.95 for vehicle) P<0.001
Wenger et al., (1997)	Sprague–Dawley rats N= 16 controls N= 17 Δ^9-THC	Daily via i.p injection on third week of gestation (GD14- day of delivery) Eval: PND 0, 5, 10, 15, 20.	Δ^9-THC: 0.02 mg/kg-day in 0.5 ml vehicle of propylene glycol and saline solution (1:1) From UN Narcotics Laboratory. Vienna, Austria Control: same volume of vehicle only	Sampling of offspring (both sexes) was done every 5th day between PND 1 - 20. Hypothalami, pituitary and gonads collected and frozen. Sera analyzed for: Gonadotropin releasing hormone, luteinizing hormone, follicle stimulating hormone, prolactin, and growth hormone	No change in behavior of pregnant rats was observed.	Δ^9-THC altered the content and production of hormones associated with the hypothalamic-pituitary axis during early development (up to PND 5). Alteration transitory - hormone profiles return to control levels by around PND 10.

Reference	Animal Model N/group	Exposure details and Timing of Evaluation	Test Agent: Doses or Concentrations	Neurodevelopmental Outcomes Assessed	Maternal Toxicity	Neurodevelopmental Toxicity
Gianutsos and Abbatiello (1972)	Wistar rats F0: N=55 pairs total Cannabis: N=21 Vehicle control: N= 21 Untreated control: N= 13 Lashley III maze N= 25 offspring (15 males and 10 females)	s.c. injection on GD 8-11 Evaluation: tested at PND 65 in a Lashley III maze	*Cannabis Sativa* extract [Mexican origin; U.S. gov seized sample]: 250 mg/kg in 2.5 ml/kg polyethylene glycol (PEG)l Vehicle Control: PEG alone Untreated control	Evaluated for: 3 out of 4 consecutive errorless trials; Number of errors committed and Time spent in the maze.	Not reported	Exposed offspring required more trials to reach 3 out of 4 consecutive errorless trials; ↑ errors in the maze and Significantly ↑ time spent in the maze. Authors reported differences were not observed when females alone were evaluated (data not provided.)

Appendix Table 4.6. Neurodevelopmental Toxicity Studies in Zebrafish

Reference	Animal Model N/group	Exposure and Terminal Evaluation	Agent, Doses or Concentrationst	Outcomes Assessed	Developmental Toxicity	Comments
Ahmed et al., (2018)	Zebrafish embryo N = 54, 48, 61, 57, and 55 for 2, 4, 6, 8 or 10 mg Δ^9-THC/L, respectively Control: methanol N = 39, 37, 22, 25, and 20 for 0.2, 0.4, 0.6, 0.8, and 1.0%, respectively Untreated Control N = 59	Δ^9-THC for 5 h during gastrulation (5.25 to 10.75 hpf) Examined at 2 and 5 days postfertilization (dpf)	Δ^9-THC at 0, 2, 4, 6, 8, or 10 mg/L [Source: Sigma]	Larva survival Heart rate, Gross morphology, Neuronal Branching, synaptic activity (nicotinic acetylcholine receptors (nAChRs expression) Stimuli responses	↓ Larva survival rate at 5dpf: 8 mg Δ^9-THC/L =31 ± 10% (P<0.05); 10 mg Δ^9-THC/L= 5 ± 5% (P<0.005) Larva hatching at 3 dpf, 75% of embryos hutched regardless of Δ^9-THC concentration) Heart rate: ↓ up to 50% compared to vehicle controls at 4 mg Δ^9-THC/L and higher concentrations (P<0.001) Body length: ↓ at 4 mg Δ^9-THC/L and higher concentrations compared to vehicle controls (P<0.001) ↑Total number of nAChR at 6 mg Δ^9-THC/L (P<0.01) ↓ response to sound stimuli in 5 dpf larvae treated with 6 mg Δ^9-THC/L (P<0.001) No effect on response to touch stimuli	
Achenbach et al. (2018)	Zebrafish larvae N=60/group, except for 0.05 µM Δ^9-THC, where N=48 (12/dose/replicate; number of replicates/dose stated to be at least three)	Behavioral assessment began 3-4 minutes after addition of Δ^9-THC and continued for approximately 3 h 20 min	Δ^9-THC at 0, 0.05, 0.1, 0.5, 1.5 or 2.0 µM [Source: Sigma-Aldrich]] Control: 0.1% methanol	Basal activity measurement for 3 h in the presence of light (approximate irradiance of 10 µmol s^{-1} m^{-2}) followed by activity measurement during a series of three 5-min dark periods separated by 5-min periods of light (also 10 µmol s^{-1} m^{-2}). Uptake kinetics	All activity compared to controls: 0.05 and 0.1 µM: Slight initial ↓ activity 0.5 and 1.5 µM: ↓ activity 2 µM: initial ↑ activity for the first 30 minutes, followed by ↓ activity Response to light/dark transitions after the initial 3 h of light: Absolute peak activity level was lower in THC group than in controls. No treatment-related differences observed when activity was normalized to the activity level during the 5 min preceding the light/dark transition, at any of the concentrations tested	Ability to assess uptake kinetics allowed for determination of actual amount of Δ^9-THC in larvae during the exposure time course and relate that to the behavioral data.

Reference	Animal Model N/group	Exposure and Terminal Evaluation	Agent, Doses or Concentrationst	Outcomes Assessed	Developmental Toxicity	Comments
Carty et al., (2018)	Tg(fli1:EGFP) zebrafish embryos from Zebrafish International Resource Center (Eugene, OR). Blastula through larval stage (2 h postfertilization [hpf] – 96 hpf) n=3 vials; 7-10 embryos/vial	Exposure to Δ^9-THC, beginning 2 hpf and continuing to 96 hpf Behavioral assessment at 96 hpf Expression of select neurogenic genes (c-fos, dazl, vasa, bdnf and reln) using RT-qPCR technology at 96 hpf	Δ^9-THC: 0, 0.3, 0.6, 1.25, 2.5 mg/L (0, 1, 2, 4, 8, µM). [Source: NIDA, USA] Control: 0.05% DMSO	Duration of movement (seconds [s]) at a velocity ≥5mm/s collected at 2 min intervals. Data analyzed on the average distance per vial (n=3).	0.3 mg/L and 0.6 mg/L exhibited a significantly ↑ duration of movement (≥5mm/s) during dark periods and 1.25 mg/l group* exhibited a significantly ↓ duration. Gene expression: ↑ in c-fos (concentration-dependent) ↑ in dazl and ↓ in vasa (not concentration-dependent) No change in bdnf or reln	*Δ^9-THC 1.25 mg/L excluded from statistical analysis due to a lack of healthy fish (n<3)
Akhtar et al. (2013)	Zebrafish larvae N = 30-36 /group Modified visual motor response test with single transition from light to dark (evaluating the transient change in the behavioral and/or locomotor activity that occurs in response to the abrupt transitions between light and dark)	Δ^9-THC exposure: Acute:1, 4, or 12 h starting at 108 hpf Chronic: 96 h starting at 24 hpf Morphological evaluation: 96 h	Locomotor activity: 0, 0.6, 1.2, 2.4 or 3.4 mg/L Δ^9-THC [Purified by centrifugal partition chromatography by the authors] (3.4 mg/L not used for 96 hour exposure) Morphological analysis: 0, 0.3, 0.6, 1.2 or 2.4 mg/L Δ^9-THC Control: 0.01% DMSO	Locomotor activity in a visual motor response test, assessed after 1, 4, 12, or 96 h exposure Morphological analysis of embryos at 5 dpf	Locomotor activity: ↑ after 1 h exposure at 0.6 ($p<0.001$), 1.2 ($p<0.01$), and 2.4 ($p<0.05$), but not 3.4 mg/L Δ^9-THC ↑ after 4 h exposure at 0.6 ($p<0.001$) and 1.2 ($p<0.01$), but not 2.4 or 3.4 mg/L Δ^9-THC ↑ after 12 h exposure at 0.6 ($p<0.001$) and 1.2 ($p<0.001$), but not 2.4 mg/L ↓ at 3.4 mg/L Δ^9-THC ($p<0.05$) Significant ↑ after 96 h only at 1.2 mg/L Δ^9-THC ($p<0.05$) Morphological analysis: ↑ incidence of pericardial edema at: 0.6 ($p<0.01$), 1.2 ($p<0.05$) and 2.4 ($p<0.05$) mg/L Δ^9-THC ↑ in incidence of yolk sac edema at: 0.3 ($p<0.001$), 0.6 ($p<0.001$), 1.2 ($p<0.001$) and 2.4 ($p<0.001$) mg/L Δ^9-THC ↑ incidence of bent body/curved primary axis at: 0.3 ($p<0.001$), 0.6 ($p<0.001$), 1.2 ($p<0.05$) and 2.4 ($p<0.05$) mg/L Δ^9-THC	Similar results in the locomotor activity test were reported for the synthetic cannabinoids WIN 55,212-2, and CP 55,940 The authors noted that the pattern of change in locomotor activity was similar to that reported in rats exposed to Δ^9-THC (i.e., dose-dependent hyperactivity followed by suppression)

Appendix 5. Epigenetics

Human Studies of Cannabis Use, with Observations of Epigenetic Effects or Observations Possibly Related to Epigenetic Effects

The studies are organized in four tables, as follows:

1) peripheral blood cell effects in cannabis users
2) germ cell effects in cannabis users
3) maternal exposure during pregnancy resulting in in utero exposure only, or
4) perinatal exposure, where exposure to the offspring occurred via lactation as well as in utero.

Within each of the tables, the studies are organized by date of publication, from most recent to oldest.

Appendix Table 5.1. Human Epigenetics: Peripheral Blood Cell Effects in Cannabis Users

eference and Study Location	Study Design, Sample Sizes, Statistical Analysis	Exposure Measurement Methods and Quantification	Outcomes of Interest	Results	Covariates/ Confounders	Comments
Gerra et al. (2018) **Baltimore City, Maryland**	Nested case-control study n=136 subjects; 40 cannabis users and 96 control subjects Subjects ages 18-60 years selected among samples collected and stored previously at the Intramural Research Program of the National Institute of Drug (Health	Methods do not specify how cannabis use (CU) was measured in the subjects CU participants defined as moderate to heavy users, with no other drug use or alcohol abuse n=20 moderate users (10-20 days in past 30 days) and n=20 heavy users	Changes in DNA methylation at seven sites of the *ANNK1*, *CBR1*, *DRD2*, and *NCAM1* genes in peripheral whole blood, using MeDIP-qPCR for quantification Potential role of gene variants affecting dopamine and cannabinoid receptors	DNA methylation was significantly higher in cannabis users compared to control subjects in two of the regions analyzed Exon 8 of *DRD2* gene at +66.7 kb from transcription start site (TSS) **(p=0.034)** and CpG region at +3 kb from TSS in the *NCAM1* gene **(p=0.0004)** No differences in DNA methylation found at *ANKK1*−0.25 kb, *DRD2* −0.4 kb and +0.9 kb, *NCAM1* +0.4 kb, *CBR1* +22.31 kb, comparing cannabis users and control subjects n logistic regression model testing the influence of genetic and environmental risk factors, considering the concurrent influence on CU of the previous significant parameters	Gender evaluated in models as a confounder Covariates included: Marital status (married vs. unmarried), Educational attainment (1. Some high school/GED, 2. High school diploma, 3. Some college, 4. College	Developmental Significance: *DRD2* and *NCAM1* play central role in dopaminergic pathway Increased methylation of *DRD2* and *NCAM1* may reflect lower mRNA expression Lower availability of *DRD2* receptors could underly reward deficit condition *NCAM1* previously implicated in functions during development and maintenance of the nervous system Limitations:

eference and Study Location	Study Design, Sample Sizes, Statistical Analysis	Exposure Measurement Methods and Quantification	Outcomes of Interest	Results	Covariates/ Confounders	Comments
	Outcomes by Neighborhood (HON)- Baltimore) Subjects from HON-Baltimore recruited among people who live in Baltimore City or one of the surrounding areas Exclusion criteria: inability to sign informed consent, age <18 years old, use of other illicit drugs or alcohol (self-report and urinary analysis) Two nested logistic regression models were used: One assessed influence of CU and sociodemographic variables, and other included genotyping data Unpaired Student's t-test	(>20 days in the last 30 days) (cited Vidot et al. 2017) Control subject were never cannabis users	function, including single nucleotide polymorphism (SNP) Taq1A (*ANNK1*) and SNP rs1049353 (*CBR1*) in peripheral whole blood Interaction of genetic variants with sociodemographic covariates	(the genetic variables rs1049353 (*CBR1*) and rs2501431 (*CBR2*), gender and educational attainment: Gender (**p<0.05**), and educational attainment (**p=0.002**) had a significant relation with CU, with males having a higher risk and levels of educational attainment higher than the reference category (some high school/GED) decreasing risk of CU Having a G allele of rs1049353 (*CBR1*) was also associated with cannabis use, compared to no use (**p=0.05**)	Graduate, 5. Masters, Ph.D.), BMI, genotyping data	Due to relatively low number of cannabis users, statistical power was limited Did not split samples based on ethnic groups, due to relatively small number of subjects per group (majority African American) Changes in DNA methylation patterns observed in peripheral cells (such as WBCs) that may not reflect changes in brain reward regions DNA methylation evaluated at single time point, and thus epigenetic changes could be due to the cannabis exposure or reflect a pre-existing condition involved in CU neurobiological vulnerability

Appendix Table 5.2. Human Epigenetics: Germ Cell Effects in Cannabis Users

Reference and Study Location	Study Design, Sample Sizes, Statistical Analysis	Exposure Measurement Methods and Quantification	Outcomes of Interest	Results	Covariates/ Confounders	Comments
Murphy et al. (2018) **Geographic location not specified** **Study in Sprague Dawley rat presented in animal table**	Cross-sectional study n=24 male, adult subjects; 12 cannabis users and 12 controls Evaluated (sperm) Initially, 61 subjects selected from 107 phone screens Exclusion criteria: nicotine/tobacco use, age, current psychiatric diagnosis, current use of prescription drugs, medical illness, lives far away, not interested Of the 61 initial recruits, there were 24 cancellations or lost to follow up, leaving 37 left for in-person screening/consent Additional exclusion criteria: urinary cotinine, carbon monoxide level, tobacco or e-cigarette use, positive urine drug screen, urinary cannabis not matching self-report Linear regression models	Self-reported cannabis over past 6 months and urinary test, performed using UPLC-MS/MS and enzyme immuno-assay (EIA). EIA has Lower Reference Limit of 25 ng/mL, can detect cannabi-noids up to five days after occasional, or up to three days of more chronic use Cannabis users self-reported ≥ weekly CU for past 6 months. Urinary levels of Δ^9-THC: 50 to 739 ng/mL (mean = 260.8, SD = 228.9 ng/mL) Creatinine adjusted THC levels: 38 to 1,628 ng/mL (mean = 329.8, SD = 460.9 ng/mL). Controls self-reported no CU in past 6 months, had Δ^9-THC urinary level of 0 ng/mL	DNA methylation in sperm using reduced representation bisulfate sequencing for quantification and validated with pyrosequencing Human dataset included 1,861,760 CpG sites. 625,262 CpG sites with missing data or <5X coverage were removed Sites were rejected if they had a calculated p-value <0.05 but a methylation difference <10% Database for Annotation, Visualization and Integrated Discovery v6.8 used to identify pathway annotations associated with differentially methylated CpG sites between groups	6,640 CpG sites differed **(p<0.05)** between cannabis users and non-users after data preprocessing and filtering Majority of CpGs (78.3%) had lower levels of methylation in user group 3,979 CpG sites differed between cannabis users and non-users by at least 10% CpG sites further restricted to those occurring within a 5,000 bp region annotated to the human reference sequence database (RefSeq), and for which there were ten 10 or more CpG sites with a significant difference between user and non-user groups, 46 genes (708 CpG sites) met criteria Maximum # CpG sites differentially methylated for given gene was for Aryl Hydrocarbon Receptor Repressor (AHRR) (94 altered CpGs, all hypomethylated by ≥10% among users) Linear regression from cannabis users (n=10) showed 183 individual CpG sites representing 177 named genes for which methylation level significantly correlated with Δ^9-THC 409 CpG sites (10.3%) significantly correlated with sperm count *PTG1R* methylation inversely correlated with Δ^9-THC level (R^2= 0.839**, p=1.97e-4**) Increased *CSNK1E* methylation associated with increased Δ^9-THC (R^2= 0.686**, p=0.003**)	None accounted for	Developmental significance: DNA methylation changes of non-imprinted genes in gametes can resist post-fertilization reprogramming and persist in the somatic cells of the offspring, including the brain, as has been shown in thousands of non-imprinted genes (Tang et al. 2015) *PTG1R*, which encodes the Prostaglandin I2 Receptor (a powerful vasodilator), associated with reduced sperm fecundity *CSNK1E*, which encodes the Casein Kinase 1 Epsilon, phosphorylates circadian clock protein PER2 and is implicated in sensitivity to opioids Limitations: Cross-sectional study design Potential confounders such as life style habits, physical condition, diet, nutrition, sleep, and alcohol use that might alter sperm DNA methylation were not accounted for Likely due to the relatively small sample size, none of the CpG site comparisons resulted in a p-value < the Bonferroni corrected p-value of $<10^{-8}$ for significance that accounts for multiple comparisons

Appendix Table 5.3. Human Epigenetics: Effects in Prenatal Exposure Studies

Reference and Study Location	Study Design, Sample Sizes, Statistical Analysis	Exposure Measurement Methods and Quantification	Outcomes of Interest	Results	Covariates/ Confounders	Comments
DiNieri et al. (2011) **Brooklyn, New York** **(Study in Long-Evans rats presented in animal table)**	Case-control study n= 25 no maternal cannabis use and n=24 maternal cannabis use Fetal brain specimens (18-22 wks gestation), were evaluated Specimens previously collected, and subset of original collection chosen based on availability of tissue at NAc level Pregnant women who elected to carry out a voluntary saline-induced abortion at Kings County Hospital from 01/2000-12/2002, recruited for study if estimated to be at mid-gestational stage of pregnancy, and fetuses included if the postmortem interval did not exceed 24 hours Multiple regression model: maternal cannabis, alcohol and cigarette use included as predictors and gene expression (*DRD1* and *DRD2*) as outcomes	Samples included in the cannabis-exposed group were based on positive maternal self-report via a questionnaire and/or maternal urine that tested positive for Δ^9-THC, and/or fetal meconium positive for Δ^9-THC Remaining samples assigned to the control group Average daily joint (ADJ) of the cannabis-exposed group calculated by converting weekly use into daily use based on yearly pattern of use For example, [seven joints/week = ADJ score of 0.90 (7 joints/week × 4 weeks/month)/ (31 days/month)] Maternal cannabis use (ADJ) = 1.24 +/- 0.2	Dopaminergic D1 (*DRD1*) and D2 (*DRD2*) mRNA transcript expression, as well as opioid peptide precursors proenkephalin (*PENK*) and prodynorphin (*PDYN*) mRNA transcript expression in the fetal nucleus accumbens (NAc), dorsal striatum, and putamen	In the fetal brain tissue, decreased *DRD2* mRNA expression in NAc in brain specimens with maternal cannabis exposure (**p=0.003**) *DRD2* mRNA levels were negatively correlated with maternal report of cannabis use (r=−0.42, **p=0.005**) *DRD2* mRNA expression was not altered in the putamen with cannabis exposure (p=0.736) *DRD1* mRNA levels were not significantly altered in the NAc with cannabis exposure (p=0.330) Although *PENK* mRNA expression tended to be slightly reduced in the cannabis group covaried for age, it was not significant since overall *PENK* alterations in the NAc (p=0.003) and dorsal striatum (p=0.003) were strongly influenced by alcohol PDYN mRNA expression was not altered with cannabis exposure (p=0.155)	Univariate statistical analyses used to study effect of each independent demographic variable (e.g. fetal age, sex, PMI, etc.) on mRNA expression; variables with p-value <0.10 included in multiple regression model Maternal cannabis, alcohol and cigarette included in all final models	Developmental significance: D2R dysregulation has been implicated in addiction risk, and thus it is plausible that cannabis could increase vulnerability to addiction and other psychiatric disorders by disrupting *DRD2* gene expression The prenatal Δ^9-THC rat model (see animal table) provided evidence that epigenetic disturbances may mediate cannabis-induced reduced *DRD2* mRNA expression, and that this impairment in gene expression could be maintained through adulthood Strengths: Target tissue from brain region obtained from human samples Rat model validated the findings Limitations: Samples from elective abortions, limiting generalizability Small study with limited statistical power

Reference and Study Location	Study Design, Sample Sizes, Statistical Analysis	Exposure Measurement Methods and Quantification	Outcomes of Interest	Results	Covariates/ Confounders	Comments
Wang et al. (2006b) **Brooklyn, New York** **(Same authors as DiNieri et al. 2011)**	Case-control study n= 21 no maternal cannabis use and n=21 maternal cannabis use Fetal brain specimens (18-22 weeks of gestation), were evaluated Fetal brain specimens previously collected, and subset of original collection chosen based on availability of tissue at NAc level Pregnant women, who had elected to carry out a voluntary saline-induced abortion at Kings County Hospital from 01/2000-12/2002, were recruited for the study if they were estimated to be at mid-gestational stage of pregnancy, and fetuses included in study if the postmortem interval did not exceed 24 hours Multiple regression	Samples included in cannabis group based on positive maternal self-report questionnaire and/or maternal urine and/or fetal meconium positive for Δ^9-THC. Remaining samples assigned to control group Based on self-report, six subjects indicated heavy Δ^9-THC intake (average daily joints greater than or equal to 0.89 joints/day, four moderate intake (average daily joints greater than or equal to 0.4 and less than 0.89 joints/day), and eight light intake (average daily joints less than 0.4 joints/day) Three subjects did not have reported drug use but were identified as Δ^9-THC-positive in meconium analysis	mRNA expression of the opioid preproproteins *PENK*, *PDYN*, and the opioid receptors (mu, kappa and delta) assessed in distinct brain regions	Gestational cannabis exposure significantly associated with: Increased mu receptor expression in the amygdala (126.7 +/- 14.0% of control value, **p=0.017**) Increase of mu receptor expression dependent on amount of reported maternal cannabis intake (r=0.40, p=**0.044**) Reduced kappa receptor mRNA in mediodorsal thalamic nucleus (56.9 +/-11.1% of control value, **p=0.034**) Reduced *PENK* expression in the caudal putamen (57.37 +/- 5.3 percent of control value, **p=0.028**) Reduction of *PENK* mRNA expression levels significantly correlated with amount of maternal cannabis intake during pregnancy (r= -0.49, **p=0.003**)	Controlled for the following potentially confounding variables in the multiple regression models: maternal alcohol and cigarette use, fetal age, sex, growth measure and post-mortem interval	Developmental significance: Opioid system plays a critical role in the regulation of emotions, reinforcement, cognition, motor function and nociception, and its alterations could have long-term impact on emotional and social behaviors through adulthood, as well as lead to the development of psychiatric illness Strengths: Included alcohol and cigarette use in the multiple regression models Limitations: Small sample size Little information on potential mechanisms by which receptor expression is being altered

Reference and Study Location	Study Design, Sample Sizes, Statistical Analysis	Exposure Measurement Methods and Quantification	Outcomes of Interest	Results	Covariates/ Confounders	Comments
Wang et al. (2004) **Brooklyn, New York** **(Same authors as DiNieri et al. 2011 and Wang et al. 2006b)**	Case-control study n= 21 no maternal cannabis use and n=21 maternal cannabis use Fetal brain specimens (18-22 weeks of gestation), were evaluated Fetal brain specimens previously collected, and subset of original collection chosen based on availability of tissue at NAc level Pregnant women, who had elected to carry out a voluntary saline-induced abortion at Kings County Hospital from 01/2000-12/2002, were recruited for the study if they were estimated to be at mid-gestational stage of pregnancy, and fetuses included in study if the postmortem interval did not exceed 24 hours Multiple regression	Samples included in cannabis group based on positive maternal self-report questionnaire and/or maternal urine and/or fetal meconium positive for Δ^9-THC. Remaining samples assigned to control group Based on self-report, six subjects indicated heavy Δ^9-THC intake (average daily joints greater than or equal to 0.89 joints/day, four moderate intake (average daily joints greater than or equal to 0.4 and less than 0.89 joints/day), and eight light intake (average daily joints less than 0.4 joints/day) Three subjects did not have reported drug use but were identified as Δ^9-THC-positive in meconium analysis	mRNA expression of the cannabinoid receptor type 1 (*CBR1*) and major dopamine receptor subtypes, (*DRD1*) and (*DRD2*) in the striatum and mesocorticolimbic structures (amygdala and hippocampus)	Specific reduction, particularly in male fetuses, of the *DRD2* mRNA expression levels in the amygdala basal nucleus in association with maternal marijuana use (54% +/- 6% reduction from control subjects in the cannabis-exposed group, **p=0.008**) Reduction positively correlated with amount of maternal marijuana intake during pregnancy (r= -.461, **p=0.005**, covariates: fetal sex, postmortem interval). When general linear model analyses performed separately on males and females, the results remained significant for male subjects only No significant cannabis-related alterations detected in the hippocampus or caudal striatum for *DRD2*, *DRD1*, and *CBR1* mRNA	Depending on the model, covariates included: postmortem interval, fetal sex, maternal alcohol and cigarette use	Developmental significance: Highlighted gender differences in response to gestational cannabis exposure The amygdala is critical for development of emotional behavior and negative mood states; also make up major component of mesocorticolimbic brain circuitry (along with the prefrontal cortex and NAc) that processed information related to reward, motivation, emotion, and cognition Strengths: Examined differences between males and females Analyzed gene receptor mRNA expression in different regions of the brain Limitations: Small sample size

Appendix Table 5.4. Human Epigenetics: Effects in Perinatal Exposure Studies

Reference and Study Location	Study Design, Sample Sizes, Statistical Analysis	Exposure Measurement Methods and Quantification	Outcomes of Interest	Results	Covariates/ Confounders	Comments
Fransquet et al. (2017) **New South Wales and Western Australia**	Nested case-control study n= 804 maternal subjects; 44 cannabis users anytime during pregnancy and 760 non-user controls) The neonates of the maternal subjects were evaluated (buccal cells) Subjects selected from longitudinal pregnancy cohort of 1,634 families (804 total neonates) recruited through general public and specialist drug and alcohol antenatal services at major hospitals in New South Wales and Western Australia Exclusion criteria: mother-infant dyads with major medical complications Statistical analysis using t-tests and multivariate linear regression models	Detailed questionnaires about drug use collected in each trimester of pregnancy and the first 8 weeks postpartum Of those who reported cannabis use, eight used cannabis daily throughout trimester 1, with the dose ranging between (the equivalent of) 0.5 and 7 joints.	DNA methylation of 19 CpG units in the dopaminergic D4 (*DRD4*) gene promoter from buccal cells collected from neonates at 8 weeks, using candidate gene approach SEQUENOM MassARRAY analysis Assay covered a 396 base pair region spanning chr11:635,510–636,905 (UCSC hg38)	Gestational cannabis use associated with increased methylation at one CpG site tested in *DRD4* (CpG.21.22.2) when adjusting for tobacco use (β + 1.48, 95% CI: 0.02-2.93, **p=0.047**) adjusted for tobacco use and regardless of other drug use; adding alcohol did not alter the association At CpG.32, weak evidence that gestational cannabis is associated with increased methylation when adjusting for other substance use (β + .67, 95% CI: -0.12-1.46**, p=0.09**) None of the associations remained significant after correction for multiple testing using a Bonferroni corrected significance level of 0.0026 given the 19 CpG units examined	Included: Tobacco use, Other substance use, Alcohol use	Strengths and validation: Buccal epithelial cells have same developmental origins to neuronal cells Prior studies provide support for buccal cells as proxy for neurodevelopmental phenotypes 97% agreement between self-report substance use and urine analysis (N= 85) Limitations: None of the associations remained significant after Bonferroni correction for multiple testing No information on timing or dose of exposure during pregnancy Only 9 cannabis users in study did not smoke tobacco Despite validity of using buccal cells, tissue specificity of DNA methylation marks may still be an issue (buccal cells vs. relevant brain tissue)

Animal Epigenetic Effects

Animal Studies of Δ^9-THC or WIN (a Model CB_1R agonist), with Observations of Epigenetic Effects, or Observations Possibly Related to Epigenetic Effects

The studies are organized in four tables, as follows:

1) Pre-conceptual exposures and parental germ cell effects
2) pre-conceptual exposures to parents only
3) maternal exposure during pregnancy resulting in in utero exposure of offspring only, or
4) perinatal exposure in which the dam was exposed during both the prenatal and postnatal period and exposure to the offspring occurred via lactation as well as in utero.

Within each of the tables, the studies are organized by chemical tested, with studies of Δ^9-THC appearing first, ordered from the most recent date of publication to oldest, and studies of WIN presented next, from most recent date of publication to oldest.

Appendix Table 5.5. Animal Epigenetics: Pre-Conceptual Exposure Studies, Parental Germ Cell Effects

Reference	Animal Model and Study Design	Exposure Methods and Dosing	Outcomes of Interest	Results	Comments
Murphy et al. (2018) **Study in humans presented in human table**	Rats, male Sprague Dawley (63 days old; sexually mature) Delta-9-tetrahydrocannabinol (Δ^9-THC) treatment for 12 days Two days after treatment, rats sacrificed and sperm	Δ^9-THC (2 mg/kg Δ^9-THC, 10% ethanol, 1% Triton X-100 in saline), or vehicle (4 mls of 10% ethanol, 1% Triton X-100 in saline) Daily oral gavage Dosing designed to represent human	DNA methylation of mature sperm (relevant to fetal epigenetic reprogramming) in F0 generation	Identified 627 genes with altered DNA methylation status in association with Δ^9-THC exposure The topmost pathways enriched with these altered genes were: 'Hippo signaling pathway' (3.6-fold enrichment; 17 genes, Bonferroni **p=0.004**), and 'Pathways in cancer' (2.3-fold enrichment; 29 genes, Bonferroni **p=0.009**) Six overlapping genes among those altered by Δ^9-THC in human (see human table) and rat sperm in the 'Hippo signaling pathway,' as well as in the 'Pathways of cancer'	DNA methylation changes of non-imprinted genes in gametes can resist post-fertilization reprogramming and persist in the somatic cells of the offspring, including the brain, as has been shown in thousands of non-imprinted genes (Tang et al. 2015) The authors speculate that the overlap between the six genes in the

Reference	Animal Model and Study Design	Exposure Methods and Dosing	Outcomes of Interest	Results	Comments
	isolated and frozen for analysis N=17 rats; N=9 vehicle only (controls) and N=8 Δ^9-THC	moderate daily Δ^9-THC use		Examined each differentially methylated CpG site for all 99 genes in common between human and rat; conservation of 39/123 CpG sites when comparing rat gene CpG sites to human genomic sequence and 51/276 when comparing human gene CpG sites to rat genomic sequence (could indicate presence of functionally relevant transcription factor binding site or enhancer element) Using UCSC Genome Browser, identified 23 genes with differentially methylated CpGs contained within the recognition sequence of one or more transcription factor binding sites Compared the 627 genes exhibiting differential DNA methylation in the rat sperm to the 473 differentially methylated genes identified from the brains of rat pups with pre-conception Δ^9-THC exposure in Watson et al. (2015) (included in this table) and found 55 overlapping genes between these two datasets, a significant enrichment **($p<0.0001$)**	'Hippo signaling pathway,' as well as in the 'Pathways of cancer' suggest that these pathways could be targets of Δ^9-THC, and if retained in the zygote, could lead to disruption in growth regulatory genes, resulting in nonviability, or increased cancer risk later in life. Limitations: Small sample size Low statistical power None of the comparisons for single CpG sites between groups significant after accounting for multiple comparisons (p-trend $<10^{-8}$)

Appendix Table 5.6. Animal Epigenetics: Pre-Conceptual Exposure Studies

Reference	Animal Model and Study Design	Exposure Methods and Dosing	Outcomes of Interest	Results	Comments
Δ^9-THC studies					
Szutorisz et al. (2016) **(*Same authors as Watson et al. (20150 and Szutorisz at al. (2014) - included in this table)**	Rats, male and female Long- Evan Δ^9-THC treatment PND 28-49 Animals mated PND 64-68 when no detectable Δ^9-THC present Mixed litters established: (PND 2): 12–14 pups from Δ^9-THC - and VEH-exposed parents, with balanced proportion of males and females/litter F1 offspring weaned (PND 24)/maintained until adolescence (PND 35) or adulthood (PND 62) 3 cohorts: each with at least 4 litters + max. 2 pups/litter	Δ^9-THC (1.5 mg/kg Δ^9-THC, 0.3% Tween 80), or vehicle (saline, 0.3% Tween) Intraperitoneal injection every third day *Exposure methods designed to mimic drug use pattern of teenagers	Expanded on the Szutorisz et al. (2014) study and studied sex-specific effects of pre-gestational Δ^9-THC, by characterizing relevant mRNA expression profiles in the NAc and dorsal striatum in the F1 generation *Genes relevant to neuropsychiatric disorders *Striatal circuitry plays essential role in behaviors related to reward processing, motivation, emotion and motor activity	In adolescence, interactions evident between treatment and sex for CB_1R **(p=0.04)**; *GRIN1* **(p=0.01)**; *GRIN2B* **(p=0.01)**, as well as a main effect of sex for *GRIN2A* **(p<0.0001)**; *GRIN2B* **(p<0.0001)**; *DLG4* **(p<0.0001)** and *DLGAP3* **(p<0.0001)**; females had higher mRNA expression levels than males reduced by Δ^9-THC In contrast, pattern of expression had no significant treatment and sex interactions; main effect of Δ^9-THC for *GRIN2B* and sex for *GRIN2A*, *GRIN2B*, *GRIA1*, *GRIAB, DLG4,* and *DLGAP3* In the adult NAc, significant main effect of Δ^9-THC detected for *GRIN2A* **(p=0.02)**, with pre-gestational Δ^9-THC leading to decreased mRNA expression; main sex effect also evident for *GRIN1*, *GRIN2A*, *GRIN2B*, *GRIA1*, *GRIA2*, *DLG4*, but no treatment and sex interactions identified In the adult dorsal striatum, decreased mRNA levels observed in offspring with pre-gestational Δ^9-THC, including *CNR1* **(p=0.04)**, *GRIN1* **(p=0.003)**, *GRIN2A* **(p=0.001)**, *GRIN2B* **(p=0.01)**, *GRIA1* **(p=0.008)**, *GRIA2* **(p=0.001)**, *DLG4* **(p=0.02)**, and *DLGAP3* **(p=0.004)** No significant interactions detected between Δ^9-THC and sex, but analysis indicated main sex effects for multiple genes, including *CNR1* **(p=0.0008)**; *GRIN2A* **(p<0.0001)**, *DLG4* **(p<0.0001)**, and *DLGAP3* **(p<0.0001)**; females expressed lower (*GRIN2A*, *DLG4*) or higher (*DLGAP3*) levels than males Strength and pattern of NAc gene correlations similar between male and female adult offspring In contrast, gene correlations with pre-conception Δ^9-THC were stronger in the dorsal striatum for the females, consistent with the locomotor disturbances only experienced by the females with pre-conception Δ^9-THC	Epigenetic mechanisms could explain differences in mRNA expression for rats with pre-conception Δ^9-THC exposure vs. control rats (see Watson et al. (2015) included in this table) For example, *GRIN2A* and *DLGAP3* contained DMRs in the rat NAc in Watson et al. (2015) Control of glutamate receptors regulated by DNA methylation (Sweatt et al. 2016) *Cnr1* encodes the CB_1R receptor, and *GRIN2A*, *DLG4*, and *DLGAP3* are glutamate receptor genes; differed expression suggests endocannabinoid involvement Both male and female reproductive tissues express CBRs and endocannabinoid ligands, and studies have demonstrated that THC can reduce sperm quality (via epigenetic mechanisms) and reduce female fertility by disrupting the ovaries [Chioccarelli et al., (2010) (included in this table) and Klonoff-Cohen et al. (2006)] Effects of cannabis on sperm and oocyte epigenome could lead to multigenerational transmission

Reference	Animal Model and Study Design	Exposure Methods and Dosing	Outcomes of Interest	Results	Comments
Watson et al. (2015) **(**Same authors as Szutorisz at al. 2016 and 2014 - included in this table)***	Rats, male and female Long- Evan Δ^9-THC treatment PND 28-49 Animals mated PND 64-68 when no detectable Δ^9-THC present, and pups raised by drug-naïve mothers N=32 pups (8 females and 8 males derived from both the Δ^9-THC and VEH-exposed lines from five to six different mothers and fathers in each group) Bilateral nucleus accumbens (NAc) tissue dissected from the frozen brains of the pups, isolated at PND 62	Δ^9-THC (1.5 mg/kg Δ^9-THC, 0.3% Tween 80), or vehicle (saline, 0.3% Tween) Intraperitoneal injection every third day *Exposure methods obtained from Szutorisz et al. (2014) (included in this table) and designed to mimic drug use pattern of teenagers	NAc genome-wide DNA methylation in the F1 generation	Identified 406 hypermethylated and 621 hypomethylated differentially methylated regions (DMRs) in F1 offspring with parental Δ^9-THC exposure, including 5611 CpGs, 3758 of which were independently significant by logistic regression **(q<0.01)** DMRs preferentially located in gene bodies (exonic and intronic regions) compared with background (UCSC rat genome browser, rn4 genome) and downstream of transcription start sites (TSS) 513 DMRs overlapped promoters or exons/intron of 492 annotated RefSeq genes Top 5 gene ontology (GO) term enrichments for DMR-associated genes included cell membrane function, animal behavior, synaptic organization, receptor activity, including proteins localized to cellular components of neurons and synapses DMRs overlapped with genes encoding regulators of synaptic plasticity and transmission, including glutamatergic-related genes such as glutamate receptors (GluRs) and kainite receptors Significant hypomethylated DMR located within first coding exon of *GRIN2A*; reanalysis of Szutorisz et al. (2014) study mRNA expression data revealed significant *GRIN2A* mRNA levels of rats with pre-conception Δ^9-THC exposure In total, 5 out of 10 DMR-associated genes in the *DLG4* network (*DLGAP2*, *KCNA5*, *BEGAIN*, *GRIN2A*, and *DLG4*) showed significant differential mRNA expression between THC and VEH groups **(P<0.05)**	NAc (ventral striatum) linked to addiction vulnerability, compulsive behaviors and reward sensitivity GluRs mediate synaptic plasticity and transmission, with impacts on addiction behavior (Szutorisz et al. 2014) Results present evidence that germline Δ^9-THC exposure → DNA methylation changes in gene loci related to synaptic plasticity and glutamatergic pathway, that resist post-fertilization reprogramming

Reference	Animal Model and Study Design	Exposure Methods and Dosing	Outcomes of Interest	Results	Comments
Szutorisz et al. (2014) **(*Same authors as Szutorisz at al. 2016 and Watson et al. (2015) - included in this table)**	Rats, male and female Long- Evans Δ^9-THC treatment PND 28-49 Animals mated PND 64-68 when no detectable Δ^9-THC present Mixed litters established: (PND 2): 12–14 pups from Δ^9-THC - and VEH-exposed parents, with balanced proportion of males and females/litter F1 offspring weaned (PND 24)/maintained until adolescence (PND 35) or adulthood (PND 62) 3 cohorts: each with at least 4 litters + max. 2 pups/litter	Δ9-THC (1.5 mg/kg Δ9-THC, 0.3% Tween 80), or vehicle (saline, 0.3% Tween) Intraperitoneal injection every third day *Exposure methods designed to mimic drug use pattern of teenagers	mRNA expression of cannabinoid, dopamine, and glutamatergic receptor genes in the NAc and striatum at PND 35 and 62 in the F1 generation Protein expression and N-methyl-D-aspartate (NMDA) receptor binding in the dorsal striatum Functional changes in synaptic plasticity Heroin self-administration *All outcomes evaluated in the male F1 offspring	Increase in mRNA expression of *CBR1* and glutamate receptors in the NAc (**p=0.03** for *CBR1*; **p=0.009** for *GRIN2A*; **p=0.04** for *GRIA2*) at the adolescent time point, in pre-conception Δ^9-THC exposed versus vehicle exposed male F1 offspring In contrast, significant decrease in mRNA expression in the dorsal striatum (**p=0.03** for *CBR1*; **p=0.009** for *DRD2*; **p=0.02** for *GRIN1*; **p=0.005** for *GRIN2A*; **p=0.03** for *GRIA1*; **p=0.03** for *GRIA2*) at the adult time point No impairments in mRNA levels of same genes studied in medial PFC and orbito-frontal cortex, brain regions that have direct connectivity with the striatum and associated with addiction vulnerability In a separate group of the F1 male offspring, membrane-bound protein level of GluN1 subunit (the product of *GRIN1* mRNA) of the NMDA receptor was decreased (p=0.04); GluN2B (the product of *GRIN2b mRNA*) also showed a consistent reduction in association with parental Δ^9-THC exposure **(p=0.02)** The activity of medium spiny neurons in the striatum is regulated by glutamatergic input, which contributes to forms of synaptic plasticity such as long term-synaptic depression (LTD); LTD strongly associated with habitual behaviors and reinforcement learning and relies on NMDA receptors and *CBR1* LTD was most prominent in the dorsal striatum, compared to the NAc, and the LTD in the former was most prominent and significantly larger with a main effect of parental treatment **(p=0.007)** in male F1 offspring. No effect detected in the NAc Parental Δ9-THC exposure associated with increased work effort to self-administer heroin, with enhanced stereotyped behaviors during period of acute heroin withdrawal	Molecular mechanisms by which parental THC exposure affects mRNA expression and phenotypes related to drug addiction and other neuropsychiatric conditions in the offspring brain remain unknown, however, epigenetic processes are likely involved (see Watson et al. (2015) included in this table) Abnormal mRNA levels in the NAc and later in the dorsal striatum mirror transition from reward-oriented to habitual, compulsive drug-taking that normally typifies progression from recreational drug use to addiction disorder (reviewed in Everitt and Robbins (2013); Gerdemanet et al. (2003))

WIN Studies					
Reference	**Animal Model and Study Design**	**Exposure Methods and Dosing**	**Outcomes of Interest**	**Results**	**Comments**
Ibn Lahmar Andaloussi et al. (2019)	Rats, male Wistar (21 days old; adolescence) Synthetic cannabinoid treatment postnatal day (PND) 30-50 (20 days) Twenty days after treatment, each male rat placed in breeding cage with two untreated females Litters culled to 7 pups on PND 1 and weaned at PND 21 N=32 pups treated with stress or no stress PND 60-68; N=8/group Pups evaluated at PND 68 and plasma and prefrontal cortex (PFC) brain tissue collected	Synthetic cannabinoid receptor 1 (CB_1R) agonist WIN 55212-2 (WIN) (1.2 mg/kg dissolved in 0.1% Tween 80 and diluted in saline 0.9%), or vehicle Daily intraperitoneal injections (10X one injection, 5X two injections, 10X no injection) Adult offspring of non-exposed fathers (NF) and offspring of WIN exposed (WF) fathers subjected to unpredictable variable stress, or no stress Dosing designed to represent human use patterns	PFC global DNA methylation in F1 generation PFC DNA methyltransferase enzyme 1 (*DNMT1*) and *DNMT3a* mRNA expression Plasma corticosterone level Novel object recognition (testing episodic-memory) and open field test (testing anxiety-like behavior) Behavioral tests for anxiety and episodic-like memory *All outcomes evaluated in F1 generation	Main effect of stress **(p< 0.001)** on global DNA methylation Significant interaction between stress and paternal exposure to WIN **(p=0.0089)** on global DNA methylation Bonferroni post-hoc analysis showed significant increase in global methylation in WF offspring, compared to NF offspring **(p<0.05)** Significant independent effect of paternal exposure to WIN **(p=0.0078)** and stress **(p=0.028)** on *DNMT1* mRNA expression Bonferroni post-hoc analysis showed increased *DNMT1* mRNA level in WF offspring, compared to NF offspring **(p<0.01)** in non-stressed animals, and increased *DNMT1* mRNA level in WF and NF offspring exposed to stress, compared to non-stressed NF offspring **(p<0.05)** Significant effect of paternal exposure to WIN on *DNMT3a* mRNA expression **(p = 0.03)** and no effect of stress Bonferroni post-hoc analysis showed increased *DNMT3a* mRNA expression in WF offspring, compared to NF offspring **(p<0.05)** Significant correlation between global DNA methylation and *DNMT1* expression **(p=0.035)**, but not between global DNA methylation and DNMT3a Increased plasma coricosterone level in WF and NF offspring exposed to stress **(p<0.001)** and no significant effect of paternal exposure to WIN Stress exposure induced significant anxiety-like behavior **(p<0.001)**, but did not affect episodic-like memory, in the WF offspring, in comparison to the NF offspring	High level of circulating corticosterone may also have contributed to increased global DNA methylation Unclear whether epigenetic changes observed in WF offspring related to disruption of paternal endocannabinoid system, or direct epigenetic modifications in sperm and/or testes that could be carried to offspring intergenerationally DNA methyltransferase enzymes are essential for epigenetic maintenance Cocaine and alcohol exposure associated with dysregulated *DNMT1* transcription in testes and sperm of adult male rodents (He et al. 2006 and Ouko et al. 2009)

Reference	Animal Model and Study Design	Exposure Methods and Dosing	Outcomes of Interest	Results	Comments
Vassolern et al. (2013)	Rats, Sprague-Dawley Maternal WIN treatment PND 30-32 At PND 60, all females (16 WIN and 16 VEH) mated with drug-naïve males At parturition, all litters culled to a size of 10 pups (5 male and 5 female), weaned on PND 21 and tested on PND 60 Only F1 female offspring studied At least six distinct litters represented within each group and a maximum of two pups from the same litter in any one group, resulting in 6-11 pups/group	Increasing doses of WIN 55,212 (WIN), or vehicle Day 1 (PND 30): 1 mg/kg Day 2 (PND 31): 2mg/kg Day 3 (PND 32): 3mg/kg Twice daily subcutaneous (s.c.) injection Morphine-induced locomotor sensitization: At PND 60, VEH-F1 and WIN-F1 females received either saline (0.9% NaCl, 1 ml/kg, s.c.) or 7.5 mg/kg morphine sulfate	mu opioid receptor (*OPRM1*) [primary morphine target], *FosB*, *cFos* [locomotor sensitization], and dopamine receptor (*DRD1*, *DRD2*) mRNA expression in the NAc in the F1 generation *OPRM1* mRNA expression in the paraventricular nucleus (PVN) Plasma corticosterone levels and corticotropin releasing hormone (CRH) mRNA expression levels from the PVN of the hypothalamus (HPA axis regulation) Morphine-induced locomotor sensitization *All outcomes evaluated in F1 female generation	Gene expression levels in the NAc examined in VEH-F1 and WIN-F1 animals 90 min after the morphine challenge (7.5 mg/kg, s.c.): Following morphine challenge, significantly higher levels of *OPRM1* **(p=0.016)** in NAc observed in WIN-F1 animals, compared to VEH-F1, but no effect of pretreatment regimen or an interaction and no changes in expression observed within the PVN Trend towards increase in expression of the FosB gene in all animals pretreated with morphine, regardless of maternal exposure (p=0.07), as well as in the expression of cFos No significant differences in *DRD1* or *DRD2* receptor gene expression in NAc, or CRH mRNA levels in the PVN When compared to VEH-F1 females pretreated with saline, WIN-F1 females treated with saline demonstrated a significantly more robust corticosterone response on the day morphine challenge was administered (p<0.05) On the day of challenge, morphine-pretreated WIN-F1 animals demonstrated a significantly enhanced response to morphine compared to morphine-pretreated VEH-F1 animals	Findings demonstrate transgenerational effects of adolescent exposure to WIN in the absence of any *in utero* exposure, which could be mediated by epigenetic mechanisms within the gametes of the dam, such as DNA methylation, posttranslational modifications to core histone protein, or differential imprinting of specific genes caused by epigenetic modifications An alternative hypothesis, is that WIN could alter the development of the reproductive axis and change the prenatal environment

Appendix Table 5.7. Animal Epigenetics: Prenatal Exposure Studies

Reference	Animal Model and Study Design	Exposure Methods and Dosing	Outcomes of Interest	Results	Comments
Gomez et al. (2003)	Rats, Wistar Maternal Δ^9-THC treatment GD 5-day before birth (GD 21) On the day before birth, mothers killed by decapitation, and their pups were removed, killed, and their brains were removed and processed At each gender and treatment condition, at least six fetuses coming from three different litters collected	Δ^9-THC (5 mg/kg weight with sesame oil), or vehicle (sesame oil alone) Daily oral dose *Dose chosen because it is an active dose to produce effects in the ontogeny of specific neuro-transmitters as revealed by prior studies	Neural adhesion molecule L1-mRNA levels in different brain structures in the F1 generation *L1 protein is a member of the immunoglobulin superfamily; plays important role in various events including cell proliferation and migration, neuritic elongation and guidance, synaptogenesis and myelinogenesis, that take place during brain development	Levels of L1 transcripts significantly increased in fetuses with prenatal Δ^9-THC exposure, compared to controls, in the fimbria **(p<0.05)**, stria terminalis **(p< 0.05)**, stria medullaris **(p< 0.01)**, and corpus callosum **(p< 0.05)** *These brain structures are all white matter regions and exhibit highly expressed cannabinoid CB_1R receptors during development Levels of L1 transcripts significantly increased in fetuses with prenatal Δ^9-THC exposure, compared to controls, in the septum nuclei ($p<0.05$) and the habenula, and trended towards an increase in the paraventricular thalamic nucleus ($p=0.118$) and the cortical subventricular zone ($p=0.105$) Levels of L1 transcripts remained unchanged in most of the grey matter structures analyzed (cerebral cortex, basolateral amygdaloid nucleus, hippocampus, remaining diencephalic structures, basal ganglia and remaining subventricular zones), as well as in a few white matter structures (fornix and fasciculus retroflexus) Post hoc analysis of the data revealed that the increases in L1-mRNA levels reached statistical significance only in the case of Δ^9-THC-exposed males, but not in the case of Δ^9-THC -exposed females	Endocannabinoid is thought to play a modulatory role in the regulation of specific processes during brain development via three potential processes, one of which involves it modulating the gene expression and/or function of neural adhesion molecules (Fernandez-Ruiz 1999 and Fernandez-Ruiz 2000) The modification of L1 in specific brain structures during the prenatal period could be a pharmacological effect related to epigenetic action that the endocannabinoid signaling system might develop during brain development

Appendix Table 5.8. Animal Epigenetics: Perinatal Exposure Studies

Reference	Animal Model and Study Design	Exposure Methods and Dosing	Outcomes of Interest	Results	Comments
DiNieri et al. (2011) **(Study in humans presented in human table)**	Rats, Long-Evans Maternal Δ^9-THC treatment GD 5- PND 2 *Treatment period corresponds to neurodevelopmental period examined in human fetal population (see human table) On PND2, litters culled 8-10 and all pups raised by vehicle-exposed dams Only males studied; two pups/litter used for different experiments Striatum tissue dissected from frozen brains of the pups, isolated at PND 2 (comparable to human fetal	Δ^9-THC (1.5 mg/kg Δ9-THC), or vehicle (saline, 0.3% Tween 80-sterile saline solution) Daily intraperitoneal injection *The dose of THC used in this paradigm is comparable to current estimates of low dose cannabis cigarettes (~16 mg of THC)	Post-translational modification of nucleosomal histone methylation in the *DRD2* locus, specifically dimethylated lysine 9 (2meH3K9) and trimethylated lysine 4 (3meH3K4) on histone H3 in the NAc of F1 offspring at PND 62 *Drd2* mRNA expression in the F1 offspring at PND 2 and PND 62 *3meH3K4 detected in transcriptionally active chromatin regions of the genome (Akbarian et al. 2009 and Black et al. 2006), while 2meH3K9 associated with repression of developmental genes	Profile of 2meH3K9 and 3meH3K4 at the *DRD2* locus in the NAc of drug-naïve (prenatally vehicle-treated) adult rats revealed higher levels of 3meH3K4 close (+0.3kb) to the TSS, and higher levels of 2meH3K9 upstream of the TSS Prenatal Δ^9-THC exposure significantly increased the repressive 2meH3K9 mark between −1.8kb (69% increase vs control) and −3kb (83% increase vs control) upstream of the TSS and decreased 3meH3K4 across the analyzed genomic fragment in the NAc at PND 62 In agreement with 3meH3K4 as a mark of transcriptional activity, its reduction was associated with decreased RNA polymerase II (Pol II) at the TSS (+0.3kb) and within the coding region(+40kb) Although no change in 2meH3K9 was observed at the *DRD1* gene, there was reduced 3meH3K4 and decreased Pol II association at this locus, despite the lack of alteration of *DRD1* transcripts in the NAc at PND 62, in the prenatal Δ^9-THC exposed group *DRD2* mRNA expression decreased by ~40% in the NAc **(p=0.001)**, but not the dorsal striatum (p=0.37), at PND 2, in the prenatal Δ^9-THC exposed group *DRD2* mRNA expression decreased by ~30% in the NAc **(p=0.001)**, but not the dorsal striatum (p=0.32), at PND 62 in the prenatal Δ^9-THC exposed group	Detection of reduced *Drd2* mRNA transcript levels in neonatal rats with prenatal Δ^9-THC exposure, together with the persistence of the change into adulthood in rats, indicated that the observed downregulation of *DRD2* gene expression may be achieved via epigenetic regulatory processes, and led authors to examine post-translational modification of nucleosomal histones in the *DRD2* locus Data suggest that the association between prenatal Δ^9-THC exposure and addiction vulnerability can be explained, at least in part, by Δ^9-THC -induced alterations in the epigenetic regulation of the *DRD2* gene in the NAc *DRD2* dysregulation implicated in addiction risk Developmental regulation of 2meH3K9 important for appropriate tissue-specific expression of a variety of gene loci not only at promoters but also in broader regulatory

Reference	Animal Model and Study Design	Exposure Methods and Dosing	Outcomes of Interest	Results	Comments
	period and PND 62 (adulthood)		in other paradigms (Horn et al. 2006 and Maze et al. 2010)	NAc core and shell, important components of motor and reward circuits respectively, dissociated at PND 62, and *DRD2* mRNA levels decreased in both Decreased DRD2 binding sites in the NAc **(p=0.05)**, but not in the dorsal striatum (p=0.782), as well as increased sensitivity to opiate reward in adulthood, at PND 62, in the prenatal Δ^9-THC exposed group	regions including enhancers (Hublitz et al. 2009 and Rentoft et al. 2008)

Appendix 6. Literature Search Results and Screening

Literature Search Results and Screening

There were 3,172 human-related studies identified from the literature search described below.

Two epidemiologists used Swift Active Screener to screen studies at the title and abstract level for inclusion in the HIM. Inclusion criteria were:

- Human study examining prenatal exposure to cannabis or Δ^9-THC as a risk factor for developmental toxicity (adverse effects on the fetus or offspring), or
- US prevalence data on cannabis use in pregnancy, or
- Information on methods of assessing exposure to cannabis in pregnant women.

Reviews (unless they were combined with meta-analyses), conference abstracts, and opinions and commentaries were excluded.

The studies included after the Swift screening process were evaluated for their ability to address the question of whether prenatal exposure to cannabis or Δ^9-THC causes developmental toxicity. The following criteria were considered in deciding to include studies, and, among those included, which were most informative:

- The study explicitly addresses exposure to cannabis smoke and/or Δ^9-THC prior to or during pregnancy/gestation as a risk factor for adverse effects in the offspring.
- Study designs deemed eligible for inclusion in the HID were prospective and retrospective cohort studies, and case-control studies (including those nested within cohort studies). While ecologic studies, case series, and case reports have value for generating hypotheses, the number of epidemiologic studies with more informative analytic designs obviated the need to include descriptive studies.
- Appropriate controls/comparison: the study included unexposed subjects who were comparable to the exposed subjects.
- Exposure to cannabis smoke or Δ^9-THC was assessed, and the methods of assessment and quantification (frequency, intensity) are described.
- The exposure of interest occurred during a susceptible period.
- Adequate exposure contrast.
- Exposure assessment included adequate assessment of prenatal cannabis use.

- Control for known potential confounders, including tobacco, alcohol and other illicit drugs.

Of the 435 references resulting from the Swift screening, studies were excluded if: they did not present results for outcomes of prenatal cannabis exposure; they assessed cannabis as a binary exposure using self-report methods, meaning participants were asked if they had used cannabis at any point during pregnancy and responded yes or no; the sample size was too small; there was no cannabis use only population; or the study did not account for co-use of tobacco, alcohol, or other illicit drugs.

From the results of this screening and other relevant studies cited within these included articles, a total of 70 studies were included for assessment of birth outcomes, and 72 studies for neurodevelopmental outcomes.

Excluded Studies

As a general rule studies were excluded for one or more of the following reasons:

- Data on cannabis exposure during pregnancy was collected as a binary measure (meaning participants responded 'yes' or 'no' regarding any cannabis use during pregnancy) without any biological assay;
- There were no cannabis-only exposure groups, or no manner of discerning the specific effect cannabis may have had on the outcome;
- The study did not control for prenatal alcohol and tobacco co-exposure;
- Extremely small sample size;
- Limited reporting of data analysis and results;
- Unclear if exposure information specific to the prenatal period;
- Univariate analysis was the only statistical method used.

Excluded studies of birth outcomes are as follows:

1. Alpert et al. 1981
2. Bottoms et al. 1982
3. Barr et al. 1984
4. Rosen et al. 1988
5. Day et al. 1989
6. Fried 1989
7. Wilcox et al. 1990
8. Witter and Niebyl 1990
9. Graham et al. 1992
10. Hernandez et al. 1992
11. Ostrea et al. 1992
12. Vaughn et al. 1993
13. Nair et al. 1994
14. Napiorkowski et al. 1996
15. Van Marter et al. 1996
16. Faden et al. 1997
17. Gurnack et al. 1997
18. Mirochnick et al. 1997
19. Ostrea et al. 1997
20. Ammenheuser et al. 1998
21. Belcher et al. 1998
22. Eyler et al. 1998
23. Fried et al. 1998
24. Green et al. 1998
25. Boskovic et al. 2001
26. D'Apolito and Hepworth 2001
27. Kukla et al. 2001
28. Frank et al. 2002
29. Visscher et al. 2003
30. Minnes et al. 2006
31. Sheih and Kravitz 2006

32. Bailey and Byrom 2007
33. Budde et al. 2007
34. Forrester and Merz 2007
35. Weinshiemer and Yanchar 2008
36. Dominici et al. 2009
37. Gargari et al. 2012
38. Hayatbakhsh et al. 2012
39. Ortigosa et al. 2012
40. Alhusen et al. 2013
41. Conradt et al. 2013
42. Heron et al. 2013
43. Bonello et al. 2014
44. Conner et al. 2015
45. Lind et al. 2015
46. Warshak et al. 2015
47. Brown et al. 2016
48. Dotters-Katz et al. 2017
49. Metz et al. 2017
50. O'Connor et al. 2017
51. Baer et al. 2018
52. Crume et al. 2018
53. Ko et al. 2018
54. Ruisch et al. 2018
55. Sharapova et al. 2018
56. Singer et al. 2018
57. Tzilos Wernette et al. 2018
58. Ashford et al. 2019
59. Luke et al. 2019

The studies of neurodevelopment that were excluded are as follows:

1. Brook et al. 1989
2. Lester et al. 1989
3. Fried 1989
4. Rodning et al. 1989
5. Astley et al. 1990
6. Hayes et al. 1991
7. Dreher et al. 1994
8. Griffith et al. 1994
9. Martin et al. 1996
10. Tronick et al. 1996
11. Singer et al. 1997
12. Eyler et al. 1998
13. Day et al. 2000
14. Richardson et al. 2000
15. Beeghly et al. 2003
16. Leech et al. 2003
17. Noland et al. 2003a
18. Noland et al. 2003b
19. Seifer et al. 2004
20. Smith et al. 2010
21. Grewen et al. 2014
22. Grewen et al. 2015
23. Salzwedel et al. 2015
24. Godleski et al 2016

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