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*Promoting Illicit Drug Prevention Initiatives Nationally*

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May 23 2019

By Email: EPSC@nt.gov.au

Submission regarding the Northern Territory Hemp Industry Bill 2019

https://parliament.nt.gov.au/__data/assets/pdf_file/0008/693278/Hemp-Industry-Bill-2019.pdf

On behalf of the Board, Drug Free Australia, please find below, current evidence that relates to the negative impact of supporting a Hemp Industry in the Northern Territory. In particular we strongly recommend that legislators consider the serious health outcomes for the Northern Territory community as a priority, rather than embarking on something simply for apparent financial gain.

In our view, based on critical current evidence, if legislators are unwilling to consider these serious concerns when introducing new or modified Northern Territory laws, they will simply duplicate the foolishness witnessed daily in overseas countries where such laws spawn negative societal consequences that far outweigh short-term profits and tax revenues.

**Please consider the following scientific evidence and current experience from other jurisdictions:**

***1.'Chronic State'*** - A documentary produced in Idaho, United States – https://vimeo.com/280127474

**2.*CBD Oil THC Analysis***: This shows that one US teaspoon (about 5 ml) of CBD oil meeting the max limit of 0.3% THC, still contains a significant amount of THC - approximately 14 mg. This amount of THC exceeds the THC limit of 10 mg per serving in CO. – **See Attachment 1 for full report.**

**3. *Farm Bill Math***: Source: The GW Pharmaceuticals' SAM presentation – **See Attachment 2**

**4. *THC dosing chart***: This is a marijuana industry publication showing what effects are expected at different mg's of THC. Note that it does not take very much to cause brain impairment.

**5. *Charlotte's Web Lab Analysis***: The amount of THC in a 30 ml bottle of Charlotte's Web, available over the Internet and supposedly "legal", has a huge 84 ml of THC in it. Further, it contains 2.8 mg of THC/ml, which closely aligns with the calculations in numbers 1 and 2 above. ***What is especially interesting is that even with this large amount of THC available in this product, the lab report states at the bottom of the second page: "This product contains less than 0.3% THC per hemp regulation."***

**6. *SDS-CBD-OIL-SERUM***: This document was used to get the specific gravity (SG) of CBD oil.

The Northern Territory Hemp Industry Bill 2019 has overlooked genotoxicity just because it is not psychoactive, please contact Albert Stuart Reece, MBBS(Hons.), FRCS(Ed.), FRCS(Glas.), FRACGP, MD Edith Cowan University Joondalup, Western Australia AUSTRALIA his email is Dr Stuart Reece asreece@bigpond.net.au he will be able to provide you the evidence regarding this very important subject.

Dr. Rich Hilderbrandt explains in his article: “I emphasize that the only distinction between marijuana and hemp is the concentration of THC. The significant point is that an extract of hemp that is not purified and monitored will certainly contain some concentration of THC”. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6140266/

**7. *Hemp and the food chain***. Hemp grown outside has the potential to cross pollinate with marijuana plants and produce large amounts of THC.

See this recent article by a Hemp farmer. His “Hemp” was producing 21% THC. https://vtdigger.org/2019/05/03/seedlings-provided-petes-greens-dispensary-pot/?fbclid=IwAR31OMq0g0vOmysJ5mKD1YkEjmMBA224c-udv21DMFk_AgYwZPETQ3jjibk

This study gives a decent idea of what would happen if hemp were used as feed. The wild cannabis growing in Pakistan probably had a THC % in the low single digits. If we believe that today’s Hemp is kept at 0.3%, that would be just one order of magnitude below the cannabis consumed by the Pakistani buffalo.

Since THC has an avidity to brain tissue, it is not too much of a jump to infer that consumers of animal products/milk from hemp fed animals would have THC affected brains, particularly children.

**The French experience**…60 times the rate of phocomelia / micromelia (no arms) in Ain and two other areas in France where hemp is legal in the food supply. Limb defects were not shown to be elevated in the present study perhaps because data for the territories, which seem to use more cannabis, was absent. Arm reduction defects were identified as being linked with prenatal cannabis exposure by the Hawaiian investigators [1]. And one also notes press reports from a birth defects registry in Ain in France near the Swiss border where a 58-fold elevation in the rate of babies born without arms was recently reported in the press together with cannabis in the food chain in Europe, and cattle born without legs. In this context it seems to be highly pertinent just a few miles away across the Swiss border there is no such elevation in the rate of phocomelia and cannabis is not allowed to be included in the food chain. Whether subsequent research in Canada will confirm such a relationship will require further data.

Hawaiian report of 2007 from Forrester. **See attached report (Selected Birth Defects w Prenatal Drug Use-Cannabis Hawaii Forrester 2007.)**

Having THC and CBD in the food chain is a very concerning issue here in Australia with our meat, fruit, fish, grains being well known as clean, especially considering the enormity of our cattle, milk and pork industries.

This link provides further evidence of the reality of potential harm if hemp becomes big business in the Northern Territory.

https://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM213153.pdf

**Kind Regards**

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Prevent.
Don't Promote Drug Use.
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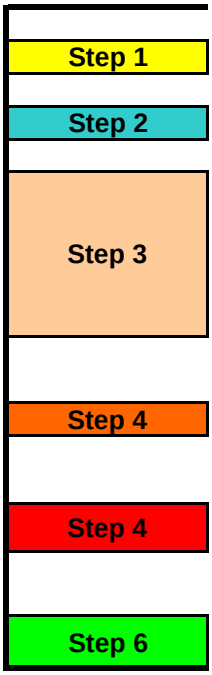


*Note 1: The Ch
*Note 2: In Colo

**Product - CBD Oil (SG Source: Safety Data Sheet for CBD Oil)**

**1 US Gallon of Water**

**1 US Gallon of CBD Oil = 8.345 lb X SG & 1 lb = 453,592 mg**

**0.3% THC weight limit in 1 US gallon of CBD oil (mg/gal)**

**mg of THC per ml based on 0.3% wt limit (1 US gal = 3785.41 ml)**

**One US teaspoon = 4.92892 ml**

**arlotte's Web Certificate of Analysis for Product Batch A00700/Product Code 910.069 lists a rado, by law, each cookie, gummy bear, etc. is limited to 10 mg of THC.**

| SG (Specific Gravity) Range | |
|---|---|
| 0.91 | 0.93 |

| | |
|---|---|
| 8.345 | lb/gal |

| MIN - lb/gal | MAX-lb/gal |
|---|---|
| 7.594 | 7.761 |

| MIN - mg/gal | MAX-mg/gal |
|---|---|
| 3,444,555 | 3,520,259 |

| MIN-mg/gal | MAX-mg/gal |
|---|---|
| 10,334 | 10,561 |

| MIN-mg/ml | MAX-mg/ml | |
|---|---|---|
| 2.7 | 2.8 | Refer to Note 1 |

| THC MIN-mg/teaspoon | THC MAX-mg/teaspoon | |
|---|---|---|
| 13.5 | 13.8 | Refer to Note 2 |

**THC content of "2.8 mg/ml".**

**The 2018 Farm Bill 0.3% "Limit" and the THC Content of Finished Products**

The 2018 Farm Bill removes "hemp" from the definition of marijuana under the federal Controlled Substances Act and therefore also deschedules hemp.

"Hemp" is defined as follows: "The term 'hemp' means the plant Cannabis sativa L. and any part of that plant, including the seeds thereof and all derivatives, ***extracts, cannabinoids***, isomers, acids, salts, and salts of isomers, whether growing or not, with a delta- 9 tetrahydrocannabinol concentration of not more than ***0.3 percent on a dry weight basis***."

When measured as a percent of the weight of a finished product, 0.3% results in products with significant amounts of THC.

**"CBD Oil"**

Vegetable oil like sesame oil weighs 920mg/ml.

Therefore, a 30ml bottle of oil will weigh 27,600mg

Multiply 27,600 by 0.003 = 82.9mg of THC

This is 2.76mg/ml of THC

**Solid CBD products:**

A 4 gram CBD gummy bear weighs 4,000mg (this is an average size gummy)

Multiply 4,000mg by 0.003 = 12mg THC

# EDIBLES DOSING CHART

| THC CONTENT PER DOSE | WHAT TO EXPECT | WHO'S IT FOR? |
|---|---|---|
| 1 - 2.5 mg THC | • Mild relief of pain, stress, anxiety, and other symptoms<br>• Improved focus and creativity | • First-time consumers<br>• Microdosers |
| 2.5 - 15 mg THC | • Stronger symptom relief<br>• Euphoria<br>• May impair coordination and alter perception | • Patients with persistent problems<br>• Restless sleepers<br>• Social butterflies |
| 15 - 30 mg THC | • Strong euphoria or unwanted effects in unaccustomed consumers<br>• May impair coordination and alter perception | • Well-seasoned consumers<br>• Medical patients with developed tolerances<br>• Experienced consumers seeking to sustain sleep |
| 30 - 50 mg THC | • Very strong euphoria in unaccustomed consumers<br>• Likely to impair coordination and alter perception | • Consumers who have poor GI absorption of cannabinoids<br>• People with significant tolerance to THC |
| 50 - 100 mg THC | • Can cause extreme side effects such as rapid heart rate, nausea, and pain<br>• Highly likely to impair coordination and alter perception | • For experienced THC individuals only<br>• Patients with cancer, inflammatory disorders, or conditions that necessitate high doses |

Always begin at the lowest recommended dose. Gradually increase by 1 or 2mg per dose, if necessary, to find your optimal dose. For more information go to Healer programs: ***www.healer.com/programs***

HEALER

Leafly

CHARLOTTE'S WEB™
STANLEY BROTHERS Boulder, CO 80301 • 719-419-8169

# CERTIFICATE OF ANALYSIS

**Product Name:** Charlotte's Web Hemp Extract Oil Maximum Strength Mint Chocolate 30mL

**Product Batch:** A00700

**Product Code:** 910.069

**Best By:** July 2020

| Parameter | Result |
|---|---|
| **Cannabinoids** *Testing performed by Eurofins Food Chemistry Testing – Boulder, CO* | |
| THC | 2.8 mg/mL |
| THC-A | 0.033 mg/mL |
| THC-V | None Detected |
| CBD | 64.3 mg/mL |
| CBD-A | 0.44 mg/mL |
| CBD-V | 0.31 mg/mL |
| CBG | 0.32 mg/mL |
| CBG-A | None Detected |
| CBN | 0.23 mg/mL |
| CBC | 2.3 mg/mL |
| Total THC per Bottle | 84 mg |
| Total THC per Serving | 1.4 mg |

**Manufactured By:** Charlotte's Web Inc.

**Manufacture Date:** 16JAN19, 18JAN19 - 20JAN19

**Batch Size:** 297,540 mL

**Units Manufactured:** 9,773

CHARLOTTE'S WEB™
STANLEY BROTHERS Boulder, CO 80301 • 719-419-8169

**Product Name:** Charlotte's Web Hemp Extract Oil Maximum Strength Mint Chocolate 30mL

**Product Batch:** A00700

**Product Code:** 910.069

**Best By:** July 2020

**Ingredient List:**

| Component | Part Number | Manufacturer | Lot Number |
|---|---|---|---|
| CO2 Extract | 500.011 | Charlotte's Web Inc. | 85.0254A |
| CO2 Extract | 500.011 | Charlotte's Web Inc. | 85.0254B |
| CO2 Extract | 500.011 | Charlotte's Web Inc. | 85.0254C |
| CO2 Extract | 500.011 | Charlotte's Web Inc. | 85.0254D |
| CO2 Extract | 500.011 | Charlotte's Web Inc. | 85.0255C |
| CO2 Extract | 500.011 | Charlotte's Web Inc. | 85.0255D |
| CO2 Extract | 500.011 | Charlotte's Web Inc. | 85.0252A |
| CO2 Extract | 500.011 | Charlotte's Web Inc. | 85.0252B |
| MCT Oil | 410.004 | CONNOILS LLC | 410.004-29AUG18 |
| MCT Oil | 410.004 | CONNOILS LLC | 410.004-11SEP18 |
| Mint Chocolate Flavoring | 410.005 | Nature's Flavors | 410.005-21SEP18 |

| **Quality Approval** | | |
|---|---|---|
| Prepared By/ Date | Approved By/ Date | Status |
| Katelyn Wuhnder — Digitally signed by Katelyn Wuhnder Date: 2019.01.30 11:43:06 -08'00' | Nicholas Morl — Digitally signed by Nicholas Morl Date: 2019.01.31 10:18:45 -07'00' | **Pass** |

This Charlotte's Web Inc.™ product has been reviewed by Quality Assurance, has been found to have met all product specifications, and is released. This product contains less than 0.3% THC per hemp regulation.

# SAFETY DATA SHEET (SDS)

**SECTION 1. IDENTIFICATION**

**IDENTITY:** CBD OIL SERUM

**MANUFACTURER'S CODE:**

| **MANUFACTURER'S NAME:** | EMERGENCY TELEPHONE: 818-466-5647 |
|---|---|
| EARTHLY BODY, INC.,<br>21900 Plummer Street<br>CHATSWORTH, CA 91311 | TELEPHONE NUMBER FOR INFORMATION: 855-745-3948 |
| | FAX NUMBER: 818-717-9334 |
| | Date Prepared : 15-Jan-17 |

**SECTION 2. HAZARDS IDENTIFICATION:**

**I. EMERGENCY OVERVIEW:**

**TYPE OF HAZARD:** Not applicable.

**APPEARANCE:**

**II. POTENTIAL HEALTH EFFECTS:**

**INHALATION:** Not applicable.
**SKIN CONTACT:** May be irritating to extra-sensitive skin.
**EYE CONTACT:** May cause mechanical irritation.
**INGESTION:** Harmful if swallowed. May cause gastrointestinal irritation.
**CHRONIC EFFECTS AND MEDICAL CONDITIONS AGGRAVATED BY OVEREXPOSURE:**
Chronic effects and medical conditions aggravated by overexposure to this product have been not established. Unnecessary exposure to this product or any chemical should be avoided.

**SECTION 3. COMPOSITION/INFORMATION**

The identity of individual components of this mixture is proprietary information and regarded to be a trade secret. The product(s) does not contain ingredients considered hazardous as defined by OSHA, 29CFR 1910.1200 and /or WHMIS under the HPA. However, based on the health hazard determination of contained ingredients present, this mixture presents the following health hazard(s):

| HAZARDOUS COMPONENTS | % RANGE | CAS NO. | OSHA PEL | ACGIH TLV |
|---|---|---|---|---|
| N/A | N/A | N/A | | |

# SAFETY DATA SHEET (SDS)

**MANUFACTURER'S NAME**: EARTHLY BODY, INC., 21900 Plummer Street CHATSWORTH CA 91311
**INFORMATION/EMERGENCY NO.** 818-466-5647
**NAME OF PRODUCTS:** CBD OIL SERUM

## SECTION 4. FIRST AID MEASURES:

**INHALATION**: Not applicable.

**SKIN:** If irritation is experienced, wash exposed area thoroughly with water. If irritation persists, get medical assistance.

**EYES:** Flush with plenty of water for 15 minutes, if irritation persists, get medical assistance.

**INGESTION:** Do not induce vomiting, drink 3-4 glasses of water. Get prompt medical attention.

## SECTION 5. FIRE FIGHTING MEASURES:

**FLAMMABILITY CLASSIFICATION:** **not flammable** **FLASH POINT: not applicable**

**EXTINGUISHING MEDIA:** Foam, $CO_2$, water fog, sand/earth.

**UNUSUAL FIRE AND EXPLOSION HAZARDS:** Not applicable.

**SPECIAL FIRE FIGHTING PROCEDURES:** Must wear NIOSH approved self-contained breathing apparatus and protective clothing. Cool fire exposed container with water spray.

## SECTION 6. ACCIDENTAL RELEASE MEASURES:

**STEPS TO BE TAKEN IN CASE MATERIAL IS RELEASED OR SPILLED:** Caution! Floor may become slippery. Absorb spilled material with inert material such as sawdust, sand or earth.

**DISPOSAL METHOD:** Sweep up and dispose of in accordance with Federal, State and local regulations.

## SECTION 7. HANDLING AND STORAGE:

**USAGE PRECAUTIONS:** Use as directed.

**STORAGE PRECAUTIONS:** Protect containers against physical damage. Keep closed until used. Store in a dry place at ambient temperatures. Keep out of reach of children.

**OTHER PRECAUTION:** N/A

# SAFETY DATA SHEET (SDS)

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**INFORMATION/EMERGENCY NO.** 818-466-5647
**NAME OF PRODUCTS:** CBD OIL SERUM

## SECTION 8. EXPOSURE CONTROLS AND PERSONAL PROTECTION:

**ENGINEERING CONTROL:** Not required.

**RESPIRATION PROTECTION:** Not applicable.

**PROTECTIVE GLOVES:** Not required.

**EYE AND FACE PROTECTION:** Not required.

**HYGIENIC WORK PRACTICES:** No special practices noted. No eating or drinking while working with this material.

## SECTION 9. PHYSICAL AND CHEMICAL PROPERTIES:

**BOILING RANGE:** Not determined.

**VAPOR DENSITY:** **X HEAVIER** _____ **LIGHTER THAN AIR**

**EVAPORATION RATE:** _____ **FASTER** **X SLOWER, THAN ETHER**

**APPEARANCE:** CLEAR OILY LIQUID

**ODOR/TASTE:** CHARACTERISTIC

**SOLUBILITY IN WATER:** NOT SOLUBLE

**pH (range):** N/A

**Specific Gravity:** 0.92+/- 0.01

**VISCOSITY (range):** Water-thin liquid

## SECTION 10. STABILITY AND REACTIVITY:

**STABILITY:** _____ **UNSTABLE** **X** **STABLE**

**INCOMPATIBILITY (MATERIALS TO AVOID):** Strong acids and oxidizing agents.

**HAZARDOUS DECOMPOSITION PRODUCTS:** None

**HAZARDOUS POLYMERIZATION:** ____ **MAY OCCUR** **X** **WILL NOT OCCUR**

**CONDITIONS TO AVOID:** Extreme temperatures.

# SAFETY DATA SHEET (SDS)

**MANUFACTURER'S NAME**: EARTHLY BODY, INC., 21900 Plummer Street CHATSWORTH CA 91311
**INFORMATION/EMERGENCY NO.** 818-466-5647
**NAME OF PRODUCTS:** CBD OIL SERUM

**SECTION 11. TOXICOLOGICAL INFORMATION:**

**HEALTH WARNING:** Not applicable.

**SECTION 12. ECOLOGICAL INFORMATION:**

Avoid uncontrolled release of this material.

**SECTION 13. DISPOSAL CONSIDERATIONS:**

**WASTE DISPOSAL METHOD:** Collect and dispose of in accordance with Federal, State and local laws and regulations. This product is not classified as an equitable hazardous waste.

**SECTION 14. TRANSPORT INFORMATION:**

**DOT CLASSIFICATION:** Not regulated.

**SECTION 15. REGULATORY INFORMATION:**

**TSCA:** Not applicable.

**SARA, Title III, Section 313 (40CFR 372):** Not applicable.

**California Proposition 65:** not applicable

**SECTION 16. OTHER INFORMATION:**

**REVISION :** N/A

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DOI: 10.1080/15287390600748799

Taylor & Francis

# Risk of Selected Birth Defects with Prenatal Illicit Drug Use, Hawaii, 1986–2002

**Mathias B. Forrester and Ruth D. Merz**
*Hawaii Birth Defects Program, Honolulu, Hawaii, USA*

**The literature on the association between prenatal illicit drug use and birth defects is inconsistent. The objective of this study was to determine the risk of a variety of birth defects with prenatal illicit drug use. Data were derived from an active, population-based adverse pregnancy outcome registry. Cases were all infants and fetuses with any of 54 selected birth defects delivered during 1986–2002. The prenatal methamphetamine, cocaine, or marijuana use rates were calculated for each birth defect and compared to the prenatal use rates among all deliveries. Among all deliveries, the prenatal use rate was 0.52% for methamphetamine, 0.18% for cocaine, and 0.26% for marijuana. Methamphetamine rates were significantly higher than expected for 14 (26%) of the birth defects. Cocaine rates were significantly higher than expected for 13 (24%) of the birth defects. Marijuana rates were significantly higher than expected for 21 (39%) of the birth defects. Increased risk for the three drugs occurred predominantly among birth defects associated with the central nervous system, cardiovascular system, oral clefts, and limbs. There was also increased risk of marijuana use among a variety of birth defects associated with the gastrointestinal system. Prenatal uses of methamphetamine, cocaine, and marijuana are all associated with increased risk of a variety of birth defects. The affected birth defects are primarily associated with particular organ systems.**

It is estimated that hundreds of thousands of women use illicit drugs during pregnancy each year in the United States (Hutchins, 1997). Studies have varied widely in the reported prevalence of illicit drug use during pregnancy due to differences in population size, population studied, and study design (Derauf et al., 2003; Norton-Hawk, 1997). Prenatal illicit drug use has been associated with preterm delivery; decreased birth weight, length, and head circumference; and adverse neurobehavioral characteristics shortly after birth, such as withdrawal symptoms (e.g., irritability, tremors, and feeding problems) (Behnke et al., 2001; Cosden et al., 1997; Holzman & Paneth, 1994; Ostrea et al., 1992; Chouteau et al., 1988; Little et al., 1988).

Studies that examine the impact of illicit drug use during pregnancy are often subject to certain limitations (Cosden et al., 1997; Hutchins, 1997; Norton-Hawk, 1997). Individuals who use one illicit drug frequently use other illicit drugs. Thus it is difficult to elicit whether the observed effects are due to a specific drug. Similarly, woman who use illicit drugs during pregnancy may also have other adverse health behaviors or inadequate prenatal care that could account for the observed outcomes.

Another difficulty is the identification of the illicit drug exposure. The two main methods for identification of illicit drug use are through self-report or through toxicology tests, neither of which is ideal. Individuals might be reluctant to report illicit drug use because of the negative moral connotations associated with the practice as well as potential legal ramifications. For the same reasons, individuals may be reluctant to undergo toxicology tests. Furthermore, toxicology tests only provide information on recent illicit drug use. Since both methods of identifying illicit drug exposure have limitations and one may not be superior to the other, it was suggested that both be used together in order to obtain a more accurate estimate of illicit drug use (Christmas et al., 1992).

A number of studies investigated whether prenatal illicit drug use causes birth defects. Various studies reported that maternal cocaine use increased risk of microcephaly, cardiac defects, situs inversus, ventricular septal defect, atrial septal defect, endocardial cushion defect, genitourinary defects, and gastroschisis (Abe et al., 2003; Ferencz et al., 1997a, 1997b, 1997c; Battin et al., 1995; Torfs et al., 1994; Lipshultz et al., 1991; Martin & Edmonds, 1991). Prenatal

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marijuana use was associated with ventricular septal defect, Ebstein anomaly, gastroschisis, and limb–body wall complex (Wlliams et al., 2004; Luehr et al., 2002; Ferencz et al., 1997e; Correa-Villasenor et al., 1994; Torfs et al., 1994). Maternal methamphetamine or amphetamine use has been reported to increase risk of cardiac defects, musculoskeletal defects, and gastroschisis (McElhatton et al., 2000; Torfs et al., 1994). However, other research observed no association between birth defects and maternal use of illicit drugs in general (Frey & Hauser, 2003; Hussain et al., 2002; Croen et al., 2000; Penman et al., 1998; Li et al., 1995), cocaine (Kuehl & Loffredo, 2002; Beaty et al., 2001; Behnke et al., 2001; Gardner et al., 1998; Ferencz et al., 1997d; Hume et al., 1997; Shaw et al., 1996; Martin & Khoury, 1992; Martin et al., 1992; Adams et al., 1989), marijuana (Steinberger et al., 2002; Beaty et al., 2001; Ferencz et al., 1997d; Shaw et al., 1996; Adams et al., 1989), or methamphetamine or amphetamine (Shaw et al., 1996; Little et al., 1988).

Much of the published research on prenatal illicit drug use and birth defects were case reports, involved a small number of cases, were not population-based, or focused on only one or a few particular birth defects. The intent of the current investigation was to evaluate the relationship between use of methamphetamine, cocaine, and marijuana during pregnancy and a variety of birth defects using population-based data from over 300,000 live births.

## METHODS

This retrospective study used data from the Hawaii Birth Defects Program (HBDP), a statewide, population-based registry for adverse pregnancy outcomes (National Birth Defects Prevention Network, 2004). The HBDP includes all infants and fetuses of any pregnancy outcome (live births, fetal deaths, and elective terminations) of any gestational age where the delivery occurred in Hawaii and a reportable birth defect, neoplasm, congenital infection, or prenatal illicit drug use was identified between conception and 1 yr after delivery. Trained HBDP staff collected information on eligible subjects through review of medical records at all delivery and tertiary care pediatric hospitals, facilities that perform elective terminations secondary to prenatal diagnosis of birth defects, genetic counseling centers, cytogenetic laboratories, and all but one of the prenatal ultrasound facilities in Hawaii. Through this multiple source system, ascertainment of infants and fetuses diagnosed with eligible conditions (at least for birth defects, neoplasm, and congenital infections) is believed to be as complete as possible because an eligible infant or fetus missed through one ascertainment source is likely to be identified through another. However, independent verification of this assertion has not been documented.

In order to select which medical records to review, the HBDP provides each health care facility with a list of International Classification of Diseases Ninth Revision (ICD-9) codes that designate conditions of interest to the HBDP. Included on this list are the ICD-9 codes for birth defects (mainly 740–759.9) and for noxious influences affecting the fetus via the placenta or breast milk (760.70–760.79). The first range of codes was used to identify infants and fetuses with birth defects, while the latter range of codes was used to identify illicit drug use during pregnancy.

A diagnosis of illicit drug use during pregnancy was based on any mention of illicit drug use during pregnancy in the medical record or a positive toxicology screen for the mother or infant during or shortly after delivery. In the HBDP database, for verification of illicit drug use a positive toxicology screen is considered to be superior to mention in the medical record. So if an illicit drug has a positive toxicology screen and is mentioned in the medical record, the HBDP database only notes that there was a positive toxicology screen. As a result, there is no way to distinguish those instances where the illicit drug use was based on both methods from those instances where the drug use was based solely on a positive toxicology screen.

Cases for the current investigation consisted of all HBDP infants and fetuses delivered during 1986–2002 with a report of prenatal illicit drug use involving methamphetamine, cocaine, or marijuana or a diagnosis of any of 54 selected birth defects. The three illicit drugs were chosen because they were the drugs most commonly reported in prenatal illicit drug use in Hawaii. The particular birth defects were chosen because they were (1) relatively common defects, (2) easy to diagnose, and/or (3) were associated with increased morbidity or mortality. These 54 birth defects are listed in Tables 1–3. All pregnancy outcomes (live births, fetal deaths, elective terminations) were included because in Hawaii a large proportion of fetuses identified with certain types of birth defects do not result in live birth (Forrester & Merz, 2004; Forrester et al., 1998).

The rate of prenatal use of methamphetamine, cocaine, and marijuana was calculated among the population using the number of live births reported to the Hawaii Department of Health as a denominator. Fetal deaths and elective terminations were not included in the denominators because it is not believed that such pregnancy outcomes are accurately reported to the Department of Health.

The rate of each of the 3 illicit drugs was then calculated for each of the 54 selected birth defects. A portion of mothers used two or more of the illicit drugs investigated during a given pregnancy. These mothers were included in all of the relevant analyses. For example, if the mother used methamphetamine and cocaine, the mother was included in the analysis of methamphetamine and the analysis of cocaine. However, in an effort to minimize confounding by associated illicit drugs, the analyses were also performed using those cases where only one of the illicit drugs was reported to have been used.

**TABLE 1**

Rate of Prenatal Methamphetamine Use Among Infants and Fetuses With Selected Birth Defects, Hawaii, 1986–2002

| Birth defect | Total cases | Total use[a] | Rate (%) | Rate ratio[b] | 95% CI[c] | Isolated use[a] | Rate (%) | Rate ratio[b] | 95% CI[c] |
|---|---|---|---|---|---|---|---|---|---|
| Anencephaly | 118 | 1 | 0.85 | 1.64 | 0.04–9.29 | 1 | 0.85 | 2.16 | 0.05–12.28 |
| Spina bifida | 144 | 0 | 0.00 | 0.00 | 0.00–5.01 | 0 | 0.00 | 0.00 | 0.00–6.62 |
| Encephalocele | 63 | 1 | 1.59 | 3.06 | 0.08–17.70 | 1 | 1.59 | 4.05 | 0.10–23.39 |
| Holoprosencephaly | 38 | 2 | 5.26 | 10.16 | 1.19–39.30 | 1 | 2.63 | 6.71 | 0.17–39.72 |
| Hydrocephaly | 353 | 5 | 1.42 | 2.73 | 0.88–6.44 | 4 | 1.13 | 2.89 | 0.78–7.47 |
| Microcephaly | 328 | 16 | 4.88 | 9.41 | 5.32–16.52 | 14 | 4.27 | 10.89 | 5.88–18.53 |
| Anophthalmia/microphthalmia | 101 | 6 | 5.94 | 11.46 | 4.11–25.83 | 3 | 2.97 | 7.58 | 1.54–22.78 |
| Cataract | 39 | 0 | 0.00 | 0.00 | 0.00–19.15 | 0 | 0.00 | 0.00 | 0.00–25.30 |
| Glaucoma | 11 | 0 | 0.00 | 0.00 | 0.00–76.90 | 0 | 0.00 | 0.00 | 0.00–101.62 |
| Anotia/microtia | 120 | 3 | 2.50 | 4.82 | 0.98–14.44 | 3 | 2.50 | 6.38 | 1.30–19.09 |
| Truncus arteriosus | 21 | 0 | 0.00 | 0.00 | 0.00–37.06 | 0 | 0.00 | 0.00 | 0.00–48.98 |
| Transposition of great arteries | 136 | 4 | 2.94 | 5.68 | 1.53–14.87 | 4 | 2.94 | 7.50 | 2.01–19.65 |
| Tetralogy of Fallot | 123 | 3 | 2.44 | 4.71 | 0.96–14.08 | 2 | 1.63 | 4.15 | 0.50–15.30 |
| Single ventricle | 28 | 2 | 7.14 | 13.79 | 1.59–54.67 | 2 | 7.14 | 18.22 | 2.10–72.24 |
| Ventricular septal defect | 1331 | 27 | 2.03 | 3.91 | 2.57–5.72 | 16 | 1.20 | 3.07 | 1.75–5.00 |
| Atrial septal defect | 686 | 16 | 2.33 | 4.50 | 2.56–7.36 | 10 | 1.46 | 3.72 | 1.78–6.88 |
| Endocardial cushion defect | 74 | 2 | 2.70 | 5.22 | 0.62–19.52 | 2 | 2.70 | 6.89 | 0.82–25.80 |
| Pulmonary valve atresia/stenosis | 293 | 3 | 1.02 | 1.98 | 0.41–5.83 | 2 | 0.68 | 1.74 | 0.21–6.35 |
| Tricuspid valve atresia/stenosis | 53 | 2 | 3.77 | 7.28 | 0.86–27.64 | 1 | 1.89 | 4.81 | 0.12–28.00 |
| Ebstein's anomaly | 16 | 1 | 6.25 | 12.06 | 0.29–77.64 | 1 | 6.25 | 15.94 | 0.38–102.61 |
| Aortic valve stenosis | 38 | 1 | 2.63 | 5.08 | 0.13–30.06 | 1 | 2.63 | 6.71 | 0.17–39.72 |
| Hypoplastic left heart syndrome | 52 | 0 | 0.00 | 0.00 | 0.00–14.19 | 0 | 0.00 | 0.00 | 0.00–18.75 |
| Coarctation of aorta | | 0 | 0.00 | 0.00 | 0.00–9.73 | 0 | 0.00 | 0.00 | 0.00–12.86 |
| Interrupted aortic arch | 14 | 0 | 0.00 | 0.00 | 0.00–58.18 | 0 | 0.00 | 0.00 | 0.00–76.89 |
| Anomalous pulmonary venous return | 43 | 0 | 0.00 | 0.00 | 0.00–17.29 | 0 | 0.00 | 0.00 | 0.00–22.85 |
| Choanal atresia/stenosis | 39 | 0 | 0.00 | 0.00 | 0.00–19.15 | 0 | 0.00 | 0.00 | 0.00–25.30 |
| Cleft palate | 228 | 8 | 3.51 | 6.77 | 2.89–13.57 | 6 | 2.63 | 6.71 | 2.44–14.84 |
| Cleft lip with/without cleft palate | 410 | 10 | 2.44 | 4.71 | 2.24–8.75 | 5 | 1.22 | 3.11 | 1.01–7.32 |
| Esophageal atresia or tracheoesophageal fistula | 69 | 1 | 1.45 | 2.80 | 0.07–16.11 | 1 | 1.45 | 3.70 | 0.09–21.29 |
| Pyloric stenosis | 255 | 4 | 1.57 | 3.03 | 0.82–7.85 | 2 | 0.78 | 2.00 | 0.24–7.30 |
| Small-intestinal atresia/stenosis | 89 | 3 | 3.37 | 6.51 | 1.32–19.64 | 2 | 2.25 | 5.73 | 0.68–21.32 |
| Anal, rectal, and large-intestinal atresia/stenosis | 162 | 3 | 1.85 | 3.57 | 0.73–10.63 | 2 | 1.23 | 3.15 | 0.38–11.56 |

(*Continued*)

**TABLE 1**
(*Continued*)

| Birth defect | Total cases | Total use[a] | Rate (%) | Rate ratio[b] | 95% CI[c] | Isolated use[a] | Rate (%) | Rate ratio[b] | 95% CI[c] |
|---|---|---|---|---|---|---|---|---|---|
| Hirschsprung's disease | 69 | 0 | 0.00 | 0.00 | 0.00–10.60 | 0 | 0.00 | 0.00 | 0.00–14.01 |
| Biliary atresia | 34 | 0 | 0.00 | 0.00 | 0.00–22.12 | 0 | 0.00 | 0.00 | 0.00–29.23 |
| Malrotation of intestines | 91 | 0 | 0.00 | 0.00 | 0.00–7.98 | 0 | 0.00 | 0.00 | 0.00–10.55 |
| Hypospadias and epispadias | 856 | 6 | 0.70 | 1.35 | 0.50–2.96 | 5 | 0.58 | 1.49 | 0.48–3.49 |
| Renal agenesis or hypoplasia | 146 | 1 | 0.68 | 1.32 | 0.03–7.48 | 0 | 0.00 | 0.00 | 0.00–6.53 |
| Cystic kidney | 144 | 1 | 0.69 | 1.34 | 0.03–7.59 | 1 | 0.69 | 1.77 | 0.05–10.03 |
| Obstructive genitourinary defect | 455 | 4 | 0.88 | 1.70 | 0.46–4.37 | 3 | 0.66 | 1.68 | 0.35–4.95 |
| Bladder exstrophy | 9 | 0 | 0.00 | 0.00 | 0.00–97.78 | 0 | 0.00 | 0.00 | 0.00–129.21 |
| Persistent cloaca | 5 | 0 | 0.00 | 0.00 | 0.00–210.61 | 0 | 0.00 | 0.00 | 0.00–278.32 |
| Congenital hip dislocation | 312 | 3 | 0.96 | 1.86 | 0.38–5.47 | 3 | 0.96 | 2.45 | 0.50–7.23 |
| Polydactyly | 568 | 11 | 1.94 | 3.74 | 1.86–6.74 | 9 | 1.58 | 4.04 | 1.84–7.73 |
| Syndactyly | 276 | 7 | 2.54 | 4.89 | 1.95–10.22 | 4 | 1.45 | 3.70 | 1.00–9.57 |
| Reduction deformity of upper limbs | 115 | 3 | 2.61 | 5.03 | 1.02–15.09 | 0 | 0.00 | 0.00 | 0.00–8.31 |
| Reduction deformity of lower limbs | 47 | 2 | 4.26 | 8.21 | 0.97–31.36 | 0 | 0.00 | 0.00 | 0.00–20.82 |
| Craniosynostosis | 159 | 0 | 0.00 | 0.00 | 0.00–4.53 | 0 | 0.00 | 0.00 | 0.00–5.99 |
| Diaphragmatic hernia | 78 | 1 | 1.28 | 2.47 | 0.06–14.20 | 0 | 0.00 | 0.00 | 0.00–12.35 |
| Omphalocele | 90 | 1 | 1.11 | 2.14 | 0.05–12.26 | 1 | 1.11 | 2.83 | 0.07–16.20 |
| Gastroschisis | 109 | 1 | 0.92 | 1.77 | 0.04–10.07 | 1 | 0.92 | 2.34 | 0.06–13.31 |
| Situs inversus | 35 | 2 | 5.71 | 11.03 | 1.29–42.93 | 2 | 5.71 | 14.57 | 1.70–56.73 |
| Trisomy 21 | 479 | 6 | 1.25 | 2.42 | 0.88–5.30 | 6 | 1.25 | 3.19 | 1.17–7.01 |
| Trisomy 13 | 62 | 0 | 0.00 | 0.00 | 0.00–11.83 | 0 | 0.00 | 0.00 | 0.00–15.64 |
| Trisomy 18 | 152 | 1 | 0.66 | 1.27 | 0.03–7.18 | 1 | 0.66 | 1.68 | 0.04–9.49 |
| Total live births | 316,508 | 1640 | 0.52 | ref | | 1241 | 0.39 | ref | |

*Note*. A delivery with more than one structural birth defect will be included in all relevant categories.

[a]Total use = all cases of methamphetamine use. Isolated use = cases of methamphetamine use excluding those cases where cocaine or marijuana were also used.

[b]Ratio of the rate of illicit drug use among birth defect cases to the rate of illicit drug use among all deliveries.

[c]CI = confidence interval.

**TABLE 2**
Rate of Prenatal Cocaine Use Among Infants and Fetuses With Selected Birth Defects, Hawaii, 1986–2002

| Birth defect | Total cases | Total use[a] | Rate (%) | Rate ratio[b] | 95% CI[c] | Isolated use[a] | Rate (%) | Rate ratio[b] | 95% CI[c] |
|---|---|---|---|---|---|---|---|---|---|
| Anencephaly | 118 | 0 | 0.00 | 0.00 | 0.00–17.85 | 0 | 0.00 | 0.00 | 0.00–30.64 |
| Spina bifida | 144 | 0 | 0.00 | 0.00 | 0.00–14.59 | 0 | 0.00 | 0.00 | 0.00–25.04 |
| Encephalocele | 63 | 0 | 0.00 | 0.00 | 0.00–33.90 | 0 | 0.00 | 0.00 | 0.00–58.19 |
| Holoprosencephaly | 38 | 0 | 0.00 | 0.00 | 0.00–57.31 | 0 | 0.00 | 0.00 | 0.00–98.37 |
| Hydrocephaly | 353 | 4 | 1.13 | 6.37 | 1.73–16.46 | 2 | 0.57 | 5.47 | 0.66–19.90 |
| Microcephaly | 328 | 2 | 0.61 | 3.43 | 0.41–12.48 | 1 | 0.30 | 2.94 | 0.07–16.51 |
| Anophthalmia/microphthalmia | 101 | 2 | 1.98 | 11.13 | 1.33–41.27 | 1 | 0.99 | 9.55 | 0.24–54.45 |
| Cataract | 39 | 1 | 2.56 | 14.41 | 0.36–85.18 | 1 | 2.56 | 24.74 | 0.61–146.22 |
| Glaucoma | 11 | 0 | 0.00 | 0.00 | 0.00–223.99 | 0 | 0.00 | 0.00 | 0.00–384.47 |
| Anotia/microtia | 120 | 0 | 0.00 | 0.00 | 0.00–17.55 | 0 | 0.00 | 0.00 | 0.00–30.12 |
| Truncus arteriosus | 21 | 0 | 0.00 | 0.00 | 0.00–107.96 | 0 | 0.00 | 0.00 | 0.00–185.31 |
| Transposition of great arteries | 136 | 2 | 1.47 | 8.27 | 0.99–30.44 | 2 | 1.47 | 14.19 | 1.70–52.26 |
| Tetralogy of Fallot | 123 | 3 | 2.44 | 13.71 | 2.79–41.02 | 1 | 0.81 | 7.85 | 0.20–44.53 |
| Single ventricle | 28 | 0 | 0.00 | 0.00 | 0.00–79.17 | 0 | 0.00 | 0.00 | 0.00–135.88 |
| Ventricular septal defect | 1331 | 20 | 1.50 | 8.45 | 5.14–13.10 | 14 | 1.05 | 10.15 | 5.53–17.09 |
| Atrial septal defect | 686 | 9 | 1.31 | 7.38 | 3.36–14.08 | 5 | 0.73 | 7.03 | 2.28–16.49 |
| Endocardial cushion defect | 74 | 0 | 0.00 | 0.00 | 0.00–28.74 | 0 | 0.00 | 0.00 | 0.00–49.32 |
| Pulmonary valve atresia/stenosis | 293 | 5 | 1.71 | 9.59 | 3.09–22.64 | 5 | 1.71 | 16.47 | 5.31–38.87 |
| Tricuspid valve atresia/stenosis | 53 | 1 | 1.89 | 10.61 | 0.26–61.71 | 1 | 1.89 | 18.21 | 0.45–105.93 |
| Ebstein's anomaly | 16 | 0 | 0.00 | 0.00 | 0.00–145.77 | 0 | 0.00 | 0.00 | 0.00–250.21 |
| Aortic valve stenosis | 38 | 0 | 0.00 | 0.00 | 0.00–57.31 | 0 | 0.00 | 0.00 | 0.00–98.37 |
| Hypoplastic left heart syndrome | 52 | 0 | 0.00 | 0.00 | 0.00–41.33 | 0 | 0.00 | 0.00 | 0.00–70394 |
| Coarctation of aorta | 75 | 2 | 2.67 | 14.99 | 1.78–56.07 | 2 | 2.67 | 25.73 | 3.06–96.25 |
| Interrupted aortic arch | 14 | 0 | 0.00 | 0.00 | 0.00–169.48 | 0 | 0.00 | 0.00 | 0.00–290.90 |
| Anomalous pulmonary venous return | 43 | 0 | 0.00 | 0.00 | 0.00–50.36 | 0 | 0.00 | 0.00 | 0.00–86.44 |
| Choanal atresia/stenosis | 39 | 0 | 0.00 | 0.00 | 0.00–55.77 | 0 | 0.00 | 0.00 | 0.00–95.73 |
| Cleft palate | 228 | 2 | 0.88 | 4.93 | 0.59–18.02 | 2 | 0.88 | 8.46 | 1.02–30.93 |
| Cleft lip with/without cleft palate | 410 | 6 | 1.46 | 8.23 | 3.00–18.06 | 2 | 0.49 | 4.71 | 0.57–17.11 |
| Esophageal atresia or tracheoesophageal fistula | 69 | 1 | 1.45 | 8.15 | 0.20–46.93 | 1 | 1.45 | 13.98 | 0.35–80.55 |
| Pyloric stenosis | 255 | 3 | 1.18 | 6.61 | 1.36–19.55 | 1 | 0.39 | 3.78 | 0.10–21.27 |

(*Continued*)

**TABLE 2**
(*Continued*)

| Birth defect | Total cases | Total use[a] | Rate (%) | Rate ratio[b] | 95% CI[c] | Isolated use[a] | Rate (%) | Rate ratio[b] | 95% CI[c] |
|---|---|---|---|---|---|---|---|---|---|
| Small-intestinal atresia/stenosis | 89 | 1 | 1.12 | 6.32 | 0.16–36.11 | 1 | 1.12 | 10.84 | 0.27–61.98 |
| Anal, rectal, and large-intestinal atresia/stenosis | 162 | 1 | 0.62 | 3.47 | 0.09–19.61 | 1 | 0.62 | 5.96 | 0.15–33.66 |
| Hirschsprung's disease | 69 | 0 | 0.00 | 0.00 | 0.00–30.87 | 0 | 0.00 | 0.00 | 0.00–52.99 |
| Biliary atresia | 34 | 1 | 2.94 | 16.53 | 0.41–98.57 | 1 | 2.94 | 28.38 | 0.70–169.18 |
| Malrotation of intestines | 91 | 0 | 0.00 | 0.00 | 0.00–23.26 | 0 | 0.00 | 0.00 | 0.00–39.92 |
| Hypospadias and epispadias | 856 | 1 | 0.12 | 0.66 | 0.02–3.67 | 1 | 0.12 | 1.13 | 0.03–6.30 |
| Renal agenesis or hypoplasia | 146 | 1 | 0.68 | 3.85 | 0.10–21.79 | 1 | 0.68 | 6.61 | 0.17–37.41 |
| Cystic kidney | 144 | 2 | 1.39 | 7.81 | 0.94–28.72 | 2 | 1.39 | 13.40 | 1.61–49.30 |
| Obstructive genitourinary defect | 455 | 4 | 0.88 | 4.94 | 1.34–12.74 | 3 | 0.66 | 6.36 | 1.30–18.71 |
| Bladder exstrophy | 9 | 0 | 0.00 | 0.00 | 0.00–284.82 | 0 | 0.00 | 0.00 | 0.00–488.88 |
| Persistent cloaca | 5 | 0 | 0.00 | 0.00 | 0.00–613.50 | 0 | 0.00 | 0.00 | 0.00–1053.04 |
| Congenital hip dislocation | 312 | 2 | 0.64 | 3.60 | 0.44–13.13 | 1 | 0.32 | 3.09 | 0.08–17.36 |
| Polydactyly | 568 | 5 | 0.88 | 4.95 | 1.60–11.62 | 5 | 0.88 | 8.49 | 2.75–19.94 |
| Syndactyly | 276 | 5 | 1.81 | 10.18 | 3.28–24.06 | 3 | 1.09 | 10.49 | 2.15–30.97 |
| Reduction deformity of upper limbs | 115 | 4 | 3.48 | 19.55 | 5.24–51.44 | 3 | 2.61 | 25.17 | 5.12–75.43 |
| Reduction deformity of lower limbs | 47 | 1 | 2.13 | 11.96 | 0.30–69.98 | 0 | 0.00 | 0.00 | 0.00–78.79 |
| Craniosynostosis | 159 | 0 | 0.00 | 0.00 | 0.00–13.20 | 0 | 0.00 | 0.00 | 0.00–22.65 |
| Diaphragmatic hernia | 78 | 1 | 1.28 | 7.21 | 0.18–41.35 | 0 | 0.00 | 0.00 | 0.00–46.73 |
| Omphalocele | 90 | 1 | 1.11 | 6.25 | 0.16–35.70 | 0 | 0.00 | 0.00 | 0.00–40.37 |
| Gastroschisis | 109 | 1 | 0.92 | 5.16 | 0.13–29.35 | 1 | 0.92 | 8.85 | 0.22–50.37 |
| Situs inversus | 35 | 0 | 0.00 | 0.00 | 0.00–62.49 | 0 | 0.00 | 0.00 | 0.00–107.26 |
| Trisomy 21 | 479 | 0 | 0.00 | 0.00 | 0.00–4.35 | 0 | 0.00 | 0.00 | 0.00–7.46 |
| Trisomy 13 | 62 | 1 | 1.61 | 9.07 | 0.23–52.42 | 1 | 1.61 | 15.56 | 0.39–89.98 |
| Trisomy 18 | 152 | 0 | 0.00 | 0.00 | 0.00–13.81 | 0 | 0.00 | 0.00 | 0.00–23.71 |
| Total live births | 316,508 | 563 | 0.18 | ref | | 328 | 0.10 | ref | |

*Note*. A delivery with more than one structural birth defect will be included in all relevant categories.

[a]Total use = all cases of cocaine use. Isolated use = cases of cocaine use excluding those cases where methamphetamine or marijuana were also used.

[b]Ratio of the rate of illicit drug use among birth defect cases to the rate of illicit drug use among all deliveries.

[c]CI = confidence interval.

**TABLE 3**

Rate of Prenatal Marijuana Use Among Infants and Fetuses With Selected Birth Defects, Hawaii, 1986–2002

| Birth defect | Total cases | Total use[a] | Rate (%) | Rate ratio[b] | 95% CI[c] | Isolated use[a] | Rate (%) | Rate ratio[b] | 95% CI[c] |
|---|---|---|---|---|---|---|---|---|---|
| Anencephaly | 118 | 0 | 0.00 | 0.00 | 0.00–12.14 | 0 | 0.00 | 0.00 | 0.00–26.66 |
| Spina bifida | 144 | 0 | 0.00 | 0.00 | 0.00–12.57 | 0 | 0.00 | 0.00 | 0.00–21.79 |
| Encephalocele | 63 | 3 | 4.76 | 18.20 | 3.66–55.68 | 3 | 4.76 | 39.98 | 8.03–122.29 |
| Holoprosencephaly | 38 | 2 | 5.26 | 20.12 | 2.35–77.85 | 1 | 2.63 | 22.09 | 0.55–130.76 |
| Hydrocephaly | 353 | 8 | 2.27 | 8.66 | 3.71–17.26 | 7 | 1.98 | 16.65 | 6.65–34.66 |
| Microcephaly | 328 | 8 | 2.44 | 9.32 | 3.99–18.59 | 5 | 1.52 | 12.80 | 4.13–30.17 |
| Anophthalmia/microphthalmia | 101 | 3 | 2.97 | 11.35 | 2.30–34.14 | 1 | 0.99 | 8.31 | 0.21–47.38 |
| Cataract | 39 | 0 | 0.00 | 0.00 | 0.00–37.92 | 0 | 0.00 | 0.00 | 0.00–83.29 |
| Glaucoma | 11 | 0 | 0.00 | 0.00 | 0.00–152.30 | 0 | 0.00 | 0.00 | 0.00–334.50 |
| Anotia/microtia | 120 | 2 | 1.67 | 6.37 | 0.76–23.52 | 2 | 1.67 | 13.99 | 1.68–51.66 |
| Truncus arteriosus | 21 | 0 | 0.00 | 0.00 | 0.00–73.41 | 0 | 0.00 | 0.00 | 0.00–161.22 |
| Transposition of great arteries | 136 | 1 | 0.74 | 2.81 | 0.07–15.93 | 1 | 0.74 | 6.17 | 0.16–34.98 |
| Tetralogy of Fallot | 123 | 3 | 2.44 | 9.32 | 1.90–27.89 | 2 | 1.63 | 13.65 | 1.64–50.37 |
| Single ventricle | 28 | 0 | 0.00 | 0.00 | 0.00–53.83 | 0 | 0.00 | 0.00 | 0.00–118.22 |
| Ventricular septal defect | 1331 | 25 | 1.88 | 7.18 | 4.63–10.65 | 14 | 1.05 | 8.83 | 4.82–14.87 |
| Atrial septal defect | 686 | 12 | 1.75 | 6.69 | 3.44–11.76 | 5 | 0.73 | 6.12 | 1.98–14.35 |
| Endocardial cushion defect | 74 | 0 | 0.00 | 0.00 | 0.00–19.54 | 0 | 0.00 | 0.00 | 0.00–42.91 |
| Pulmonary valve atresia/stenosis | 293 | 5 | 1.71 | 6.52 | 2.10–16.40 | 4 | 1.37 | 11.46 | 3.10–29.66 |
| Tricuspid valve atresia/stenosis | 53 | 1 | 1.89 | 7.21 | 0.18–41.96 | 0 | 0.00 | 0.00 | 0.00–60.52 |
| Ebstein's anomaly | 16 | 0 | 0.00 | 0.00 | 0.00–99.12 | 0 | 0.00 | 0.00 | 0.00–217.69 |
| Aortic valve stenosis | 38 | 1 | 2.63 | 10.06 | 0.25–59.54 | 1 | 2.63 | 22.09 | 0.55–130.76 |
| Hypoplastic left heart syndrome | 52 | 2 | 3.85 | 14.70 | 1.74–55.85 | 2 | 3.85 | 32.29 | 3.81–122.65 |
| Coarctation of aorta | 75 | 1 | 1.33 | 5.10 | 0.13–29.28 | 1 | 1.33 | 11.19 | 0.28–64.30 |
| Interrupted aortic arch | 14 | 0 | 0.00 | 0.00 | 0.00–115.24 | 0 | 0.00 | 0.00 | 0.00–253.09 |
| Anomalous pulmonary venous return | 43 | 0 | 0.00 | 0.00 | 0.00–34.24 | 0 | 0.00 | 0.00 | 0.00–75.20 |
| Choanal atresia/stenosis | 39 | 0 | 0.00 | 0.00 | 0.00–37.92 | 0 | 0.00 | 0.00 | 0.00–83.29 |
| Cleft palate | 228 | 6 | 2.63 | 10.06 | 3.65–22.24 | 4 | 1.75 | 14.73 | 3.98–38.23 |
| Cleft lip with/without cleft palate | 410 | 7 | 1.71 | 6.53 | 2.61–13.57 | 4 | 0.98 | 8.19 | 2.22–21.13 |
| Esophageal atresia or tracheoesophageal fistula | 69 | 0 | 0.00 | 0.00 | 0.00–20.99 | 0 | 0.00 | 0.00 | 0.00–46.11 |
| Pyloric stenosis | 255 | 5 | 1.96 | 7.50 | 2.41–17.72 | 4 | 1.57 | 13.17 | 3.56–34.13 |
| Small-intestinal atresia/stenosis | 89 | 2 | 2.25 | 8.59 | 1.02–31.95 | 1 | 1.12 | 9.43 | 0.24–53.93 |
| Anal, rectal, and large-intestinal atresia/stenosis | 162 | 3 | 1.85 | 7.08 | 1.46–21.06 | 2 | 1.23 | 10.36 | 1.25–38.05 |
| Hirschsprung's disease | 69 | 0 | 0.00 | 0.00 | 0.00–20.99 | 0 | 0.00 | 0.00 | 0.00–46.11 |
| Biliary atresia | 34 | 0 | 0.00 | 0.00 | 0.00–43.81 | 0 | 0.00 | 0.00 | 0.00–96.21 |
| Malrotation of intestines | 91 | 1 | 1.10 | 4.20 | 0.11–24.00 | 1 | 1.10 | 9.23 | 0.23–52.71 |

(*Continued*)

**TABLE 3**
(*Continued*)

| Birth defect | Total cases | Total use[a] | Rate (%) | Rate ratio[b] | 95% CI[c] | Isolated use[a] | Rate (%) | Rate ratio[b] | 95% CI[c] |
|---|---|---|---|---|---|---|---|---|---|
| Hypospadias and epispadias | 856 | 4 | 0.47 | 1.79 | 0.49–4.59 | 3 | 0.35 | 2.94 | 0.61–8.63 |
| Renal agenesis or hypoplasia | 146 | 2 | 1.37 | 5.24 | 0.63–19.26 | 1 | 0.68 | 5.75 | 0.15–32.55 |
| Cystic kidney | 144 | 1 | 0.69 | 2.65 | 0.07–15.03 | 1 | 0.69 | 5.83 | 0.15–33.00 |
| Obstructive genitourinary defect | 455 | 7 | 1.54 | 5.88 | 2.35–12.22 | 5 | 1.10 | 9.23 | 2.98–21.69 |
| Bladder exstrophy | 9 | 0 | 0.00 | 0.00 | 0.00–193.66 | 0 | 0.00 | 0.00 | 0.00–425.34 |
| Persistent cloaca | 5 | 0 | 0.00 | 0.00 | 0.00–417.15 | 0 | 0.00 | 0.00 | 0.00–916.18 |
| Congenital hip dislocation | 312 | 1 | 0.32 | 1.23 | 0.03–6.88 | 0 | 0.00 | 0.00 | 0.00–9.99 |
| Polydactyly | 568 | 8 | 1.41 | 5.38 | 2.31–10.68 | 6 | 1.06 | 8.87 | 3.24–19.42 |
| Syndactyly | 276 | 13 | 4.71 | 18.00 | 9.47–31.30 | 8 | 2.90 | 24.33 | 10.40–48.63 |
| Reduction deformity of upper limbs | 115 | 7 | 6.09 | 23.27 | 9.15–49.50 | 3 | 2.61 | 21.90 | 4.45–65.63 |
| Reduction deformity of lower limbs | 47 | 3 | 6.38 | 24.40 | 4.86–75.80 | 0 | 0.00 | 0.00 | 0.00–68.55 |
| Craniosynostosis | 159 | 0 | 0.00 | 0.00 | 0.00–8.97 | 0 | 0.00 | 0.00 | 0.00–19.71 |
| Diaphragmatic hernia | 78 | 0 | 0.00 | 0.00 | 0.00–18.51 | 0 | 0.00 | 0.00 | 0.00–40.66 |
| Omphalocele | 90 | 1 | 1.11 | 4.25 | 0.11–24.27 | 0 | 0.00 | 0.00 | 0.00–35.13 |
| Gastroschisis | 109 | 3 | 2.75 | 10.52 | 2.14–31.57 | 3 | 2.75 | 23.11 | 4.69–69.34 |
| Situs inversus | 35 | 1 | 2.86 | 10.92 | 0.27–64.98 | 1 | 2.86 | 23.99 | 0.59–142.71 |
| Trisomy 21 | 479 | 3 | 0.63 | 2.39 | 0.49–7.04 | 3 | 0.63 | 5.26 | 1.08–15.46 |
| Trisomy 13 | 62 | 0 | 0.00 | 0.00 | 0.00–23.43 | 0 | 0.00 | 0.00 | 0.00–51.47 |
| Trisomy 18 | 152 | 0 | 0.00 | 0.00 | 0.00–9.39 | 0 | 0.00 | 0.00 | 0.00–20.62 |
| Total live births | 316,508 | 828 | 0.26 | ref | | 377 | 0.12 | ref | |

*Note*. A delivery with more than one structural birth defect will be included in all relevant categories.

[a]Total use = all cases of marijuana use. Isolated use = cases of marijuana use excluding those cases where methamphetamine or cocaine were also used.

[b]Ratio of the rate of illicit drug use among birth defect cases to the rate of illicit drug use among all deliveries.

[c]CI = confidence interval.

The illicit drug use rates among the birth defects were then compared to the rate among all births by calculating the rate ratio and 95% confidence interval (CI) using Poisson probability.

## RESULTS

The HBDP identified 1640 cases of prenatal methamphetamine use, 563 cases of prenatal cocaine use, and 829 cases of prenatal marijuana use among deliveries during 1986–2002. During the same time period, there were 316,508 live births reported in Hawaii. Thus the prenatal use rate was 0.52% for methamphetamine, 0.18% for cocaine, and 0.26% for marijuana. If cases where 2 or more of the illicit drugs were used are excluded, there were 1241 cases of prenatal methamphetamine use, 328 cases of prenatal cocaine use, and 377 cases of prenatal marijuana use. The prenatal use rates for isolated exposures were then 0.39% for methamphetamine, 0.10% for cocaine, and 0.12% for marijuana.

During this 17-yr time period, there were 7293 infants and fetuses with one or more of the 54 birth defects of interest. Of these cases, 6545 (89.7%) were live births, 207 (2.8%) fetal deaths, 527 (7.2%) elective terminations, and 14 (0.2%) unknown pregnancy outcome. The live birth rate varied from 16.1% for anencephaly to 100% for cataract, glaucoma, interrupted aortic arch, choanal atresia/stenosis, Hirschsprung's disease, persistent cloaca, and craniosynostosis.

Table 1 contains the prenatal methamphetamine use rate among selected birth defects. Prenatal methamphetamine rates were significantly higher than expected for 14 (26%) of the birth defects. Most of these defects involved the central nervous system (holoprosencephaly, microcephaly), cardiovascular system (transposition of great arteries, single ventricle, ventricular septal defect, atrial septal defect), oral clefts (cleft palate alone, cleft lip with/without cleft palate), and limbs (polydactyly, syndactyly, reduction deformity of upper limbs). Other birth defects with significantly higher than expected prenatal methamphetamine rates were anophthalmia/microphthalmia, small-intestinal atresia/stenosis, and situs inversus. If the analysis was restricted only to those cases where methamphetamine alone was used, then the rates were significantly higher than expected for 12 (22%) of the birth defects (microcephaly, anophthalmia/microphthalmia, anotia/microtia, transposition of great arteries, single ventricle, ventricular septal defect, atrial septal defect, cleft palate alone, cleft lip with/without cleft palate, polydactyly, situs inversus, trisomy 21).

Table 2 presents the prenatal cocaine use rate for the same birth defects. Prenatal cocaine rates were significantly higher than expected for 13 (24%) of the birth defects. These defects were primarily associated with the central nervous system (hydrocephaly), cardiovascular system (tetralogy of Fallot, ventricular septal defect, atrial septal defect, pulmonary valve atresia/stenosis, coarctation of aorta), oral clefts (cleft lip with/without cleft palate), and limbs (polydactyly, syndactyly, reduction deformity of upper limbs). Other birth defects with significantly higher than expected cocaine rates were anophthalmia/microphthalmia, pyloric stenosis, and obstructive genitourinary defect. If the analysis included only the cases where cocaine alone was reported, then the rates were significantly higher than expected for 11 (20%) of the birth defects (transposition of great arteries, ventricular septal defect, atrial septal defect, pulmonary valve atresia/stenosis, coarctation of aorta, cleft palate alone, cystic kidney, obstructive genitourinary defect, polydactyly, syndactyly, reduction deformity of upper limbs).

Table 3 shows the prenatal marijuana use rate for the 54 birth defects. Prenatal marijuana rates were significantly higher than expected for 21 (39%) of the birth defects. The birth defects with greater than expected prenatal marijuana use rates were mainly defects of the central nervous system (encephalocele, holoprosencephaly, hydrocephaly, microcephaly), cardiovascular system (tetralogy of Fallot, ventricular septal defect, atrial septal defect, pulmonary valve atresia/stenosis, hypoplastic left heart syndrome), oral clefts (cleft palate alone, cleft lip with/without cleft palate), gastrointestinal system (pyloric stenosis, small-intestinal atresia/stenosis, anal/rectal/large-intestinal atresia/stenosis), and limbs (polydactyly, syndactyly, reduction deformity of upper limbs, reduction deformity of lower limbs). Other birth defects with significantly increased prenatal marijuana rates were anophthalmia/microphthalmia, obstructive genitourinary defect, and gastroschisis. If the analysis was limited to those cases where marijuana by itself was used, then the rates were significantly higher than expected for 19 (35%) of the birth defects (encephalocele, hydrocephaly, microcephaly, anotia/microtia, tetralogy of Fallot, ventricular septal defect, atrial septal defect, pulmonary valve atresia/stenosis, hypoplastic left heart syndrome, cleft palate alone, cleft lip with/without cleft palate, pyloric stenosis, anal/rectal/large-intestinal atresia/stenosis, obstructive genitourinary defect, polydactyly, syndactyly, reduction deformity of upper limbs, gastroschisis, trisomy 21).

## DISCUSSION

Using data from a statewide, population-based registry that covered over 300,000 births and a 17-yr period, this investigation examined the association between over 50 selected birth defects and maternal use of methamphetamine, cocaine, or marijuana during pregnancy. Much of the literature on prenatal illicit drug use and birth defects involved case reports, involved a small number of cases, were not population-based, or focused on only one or a few particular birth defects.

There are various limitations to this investigation. The number of cases for many of the birth defects categories was relatively small, limiting the ability to identify statistically significant differences and resulting in large confidence intervals. In spite of this, a number of statistically significant analyses were identified. Some statistically significant results might

be expected to occur by chance. If 1 in every 20 analyses is expected to result in statistically significant differences solely by chance, then among the 162 analyses performed in this study, 8 would be expected to be statistically significant by chance. However, 48 statistically significant differences were identified. Thus, not all of the statistically significant results are likely to be due to chance.

This study included all pregnancies where methamphetamine, cocaine, or marijuana use was identified through either report in the medical record or positive toxicology test. This was done because neither self-report nor toxicology testing is likely to identify all instances of prenatal illicit drug use (Christmas et al., 1992). In spite of using both methods for determining prenatal illicit drug use, all pregnancies involving methamphetamine, cocaine, or marijuana were not likely to have been identified. The degree of under ascertainment is unknown. A previous study examined the maternal drug use rate around the time of delivery in Hawaii during 1999 (Derauf et al., 2003). This study found 1.4% of the pregnancies involved methamphetamine use and 0.2% involved marijuana use. Among 1999 deliveries, the HBDP identified a prenatal methamphetamine use rate of 0.7% and a marijuana use rate of 0.4%. However, comparisons between the 2 studies should be made with caution because the previous study collected data from a single hospital during only a 2-mo period.

Another limitation is that the present study did not control for potential confounding factors such as maternal demographic characteristics, health behaviors, and prenatal care. Increased risk of birth defects has been associated with inadequate prenatal care (Carmichael et al., 2002), maternal smoking (Honein et al., 2001), and maternal alcohol use (Martinez-Frias et al., 2004). These factors are also found with maternal illicit drug use (Cosden et al., 1997; Hutchins, 1997; Norton-Hawk, 1997). Thus the increased risk of selected birth defects with illicit drug use in this study might actually be due to one of these other underlying factors. Unfortunately, information on some of the potential confounding factors such as socioeconomic status are not collected by the HBDP. Information collected on some other factors such as smoking and alcohol use is suspect because of negative attitudes toward their use during pregnancy. Moreover, the small number of cases among many of the birth defects groups would make controlling for these factors difficult.

Finally, this investigation included use of the illicit drugs at any time during the pregnancy. Most birth defects are believed to occur at 3–8 wk after conception (Makri et al., 2004; Sadler, 2000). In a portion of the cases, the drug use might have occurred at a time when it could not have caused the birth defect. Furthermore, this study does not include information on dose; however, teratogenicity of a substance may depend on its dose (Werler et al., 1990). In spite of the various potential concerns of the present study, data may suggest future areas of investigation where the limitations inherent in the present one are excluded.

This investigation found significantly higher than expected rates for prenatal use of methamphetamine, cocaine, and marijuana among a number of specific birth defects. Although not identical, there were general similarities between the three illicit drugs and the birth defects with which they were associated. Increased rates for methamphetamine, cocaine, and marijuana occurred predominantly among birth defects affecting the central nervous system, cardiovascular system, oral clefts, and limbs. There were also increased rates of marijuana use with a variety of birth defects associated with the gastrointestinal system. With the exception of marijuana and encephalocele, none of illicit drugs were associated with neural-tube defects (anencephaly, spina bifida, encephalocele). The rates of use for the three illicit drugs were not significantly elevated with eye defects other than anophthalmia/microphthalmia, genitourinary defects, and musculoskeletal defects aside from limb defects. In the majority of instances, the associations between particular illicit drugs and birth defects were found whether or not those cases involving use of multiple types of drugs were included. Of the 14 significant associations between methamphetamine and specific birth defects, 10 (71.4%) remained once multiple drug cases were excluded. Corresponding rates were 61.5% (8 of 13) for cocaine and 81.0% (17 of 21) for marijuana.

The similarities in the patterns of birth defects with which methamphetamine, cocaine, and marijuana are associated might suggest that the three drugs exert similar effects on embryonic and fetal development. This might not be expected, considering that the three illicit drugs differ in their mechanisms of action and clinical effects (Leiken & Paloucek, 1998).

Some of the associations between methamphetamine, cocaine, and marijuana observed in the present investigation were previously reported. Other studies observed similar associations, or lack thereof, of methamphetamine or amphetamine with neural-tube defects (Shaw et al., 1996) and cardiovascular and musculoskeletal defects (McElhatton et al., 2000); cocaine with neural-tube defects (Shaw et al., 1996), cardiovascular defects (Lipshultz et al., 1991), ventricular septal defect and atrial septal defect (Ferencz et al., 1997c; Martin & Edmonds, 1991), tricuspid atresia (Ferencz et al., 1997d), craniosynostosis (Gardner et al., 1998), and situs inversus (Kuehl & Loffredo, 2002); and marijuana with neural-tube defects (Shaw et al., 1996), single ventricle (Steinberger et al., 2002), ventricular septal defect (Williams et al., 2004), tricuspid atresia (Ferencz et al., 1997d), and gastroschisis (Torfs et al., 1994).

In contrast, this study differed from other research with respect to their findings regarding methamphetamine or amphetamine and gastroschisis (Torfs et al., 1994); cocaine and microcephaly (Martin & Edmonds, 1991), conotruncal defects (Adams et al., 1989), endocardial cushion defect (Ferencz et al., 1997b), situs inversus (Ferencz et al., 1997a), oral clefts (Beaty et al., 2001), and genitourinary defects (Abe et al., 2003; Battin et al., 1995; Martin & Edmonds, 1991); and marijuana and conotruncal defects (Adams et al., 1989), Ebstein anomaly (Ferencz et al., 1997e; Correa-Villasenor et al., 1994), and oral

clefts (Beaty et al., 2001). The inconsistent findings between this and the other studies could be due to differences in study methodology, case classification, or number of cases.

The mechanisms by which methamphetamine, cocaine, and marijuana might contribute to the rates for birth defects is currently unknown. Any potential explanation would have to take into account the observation that each of the illicit drugs was associated with a variety of specific birth defects affecting different organ systems. This might suggest that these three drugs would need to influence a basic, common factor involved in embryonic development.

Folic acid is involved in nucleic acid synthesis and cellular division (Scholl & Johnson, 2000) and thus would play an important role in the early growth and cellular proliferation of the embryo. Folic acid has been found to prevent a variety of birth defects (Forrester & Merz, 2005). Thus, anything that interferes with the activity of folic acid might be expected to increase the risk for these birth defects. Many of these birth defects were associated with methamphetamine, cocaine, and/or marijuana in the present study. However, two of the birth defects most closely affected by folic acid—anencephaly and spina bifida—were not associated with any of the three illicit drugs.

Vascular disruption has been suggested as a potential cause for a variety of different birth defects such as intestinal atresia/stenosis, limb reduction defects, and gastroschisis. Since cocaine is a vasoconstrictor, it has been hypothesized that cocaine use could increase the risk of these vascular disruption defects (Hume et al., 1997; Martin et al., 1992; Hoyme et al., 1983; de Vries, 1980). Although this investigation found an association between cocaine and limb reduction deformities, no association was found with intestinal atresia/stenosis or gastroschisis.

In conclusion, this study found that prenatal use of methamphetamine, cocaine, or marijuana were associated with increased risk of a variety of birth defects. The affected birth defects were primarily associated with particular organ systems. Because of various limitations of the study, further research is recommended.

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# *Cannabis sativa* (Hemp) Seeds, $\Delta^9$-Tetrahydrocannabinol, and Potential Overdose

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## Abstract

**Introduction:** *Cannabis sativa* (hemp) seeds are popular for their high nutrient content, and strict regulations are in place to limit the amount of potentially harmful phytocannabinoids, especially $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC). In Canada, this limit is 10 $\mu$g of $\Delta^9$-THC per gram of hemp seeds (10 ppm), and other jurisdictions in the world follow similar guidelines.

**Materials and Methods:** We investigated three different brands of consumer-grade hemp seeds using four different procedures to extract phytocannabinoids, and quantified total $\Delta^9$-THC and cannabidiol (CBD).

**Discussion:** We discovered that $\Delta^9$-THC concentrations in these hemp seeds could be as high as 1250% of the legal limit, and the amount of phytocannabinoids depended on the extraction procedure employed, Soxhlet extraction being the most efficient across all three brands of seeds. $\Delta^9$-THC and CBD exhibited significant variations in their estimated concentrations even from the same brand, reflecting the inhomogeneous nature of seeds and variability due to the extraction method, but almost in all cases, $\Delta^9$-THC concentrations were higher than the legal limit. These quantities of total $\Delta^9$-THC may reach as high as 3.8 mg per gram of hemp seeds, if one were consuming a 30-g daily recommended amount of hemp seeds, and is a cause for concern for potential toxicity. It is not clear if these high quantities of $\Delta^9$-THC are due to contamination of the seeds, or any other reason.

**Conclusion:** Careful consideration of the extraction method is very important for the measurement of cannabinoids in hemp seeds.

**Keywords:** cannabidiol; *Cannabis sativa* seeds; hemp seeds; overdose; phytocannabinoid extraction; tetrahydrocannabinol

## Introduction

*Cannabis* spp. of plants produce a unique class of compounds called cannabinoids. Hemp is a variety of the *Cannabis sativa* plant species that is grown specifically for the industrial uses of its derived products.[1–3] This plant can be refined into a variety of commercial items, including food, and animal feed. *C. sativa* species leads to both medical cannabis and industrial hemp, and this species contains the psychoactive component $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC); these two plants are two distinct strains with unique phytochemical signatures.[1] Hemp has lower concentrations of $\Delta^9$-THC, thus limiting its psychoactive effects, and its concentration is regulated in the consumer products where hemp is legal.[4,5] The seeds of hemp are rich in unsaturated fats and protein, while containing little to no cholesterol. In fact, a 100 g serving of seeds meets up to 63% of the recommended daily value for protein.[6] Whether in the raw seed form or as a derived product such as cold-pressed seed oil, hemp seeds have become increasingly popular as both food and health supplements; in 2011, the United States alone spent more

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than $11 million on hemp imports for consumption. In most nutritional food stores and grocery stores, hemp seeds are a staple nowadays, in countries where it is legal.

Hemp seeds produce negligible, if any, quantities of THC endogenously.[7] While food-grade strains of hemp must contain less than 0.3% $\Delta^9$-THC by weight (whole plant), they may not be free of this compound entirely. During the harvesting process, hemp seeds may become contaminated by material from other parts of the plant (such as the $\Delta^9$-THC-rich trichomes on flowers) and thus acquire $\Delta^9$-THC onto their outer shells.[7] Exposure to high concentrations of $\Delta^9$-THC could lead to psychological events and gastrointestinal disorders, including acute toxic events such as sedation. In Switzerland, four patients suffered psychological and gastrointestinal issues due to consumption of hemp seed oil, which had higher concentrations of $\Delta^9$-THC, prompting public health inquiry.[8] A recent case of $\Delta^9$-THC poisoning was reported in a toddler who was on a prescription of hemp seed oil to strengthen the immune system.[9] The toddler exhibited symptoms such as stupor and low stimulatability, which are characteristic of $\Delta^9$-THC intoxication.

In Canada, the $\Delta^9$-THC content of hemp products is tightly regulated.[5] The Industrial Hemp Regulation (IHR) Program only permits the importation, exportation, sale, and provision of hemp seeds and its derivatives that contain less than 10 $\mu$g of THC per gram of food-grade hemp seeds for consumption.[5] Products that exceed this threshold are regulated similar to medical cannabis under the Controlled Drugs and Substances Act, under Narcotics Control Regulations with strict monitoring.[10]

We were interested in investigating various chemical procedures that one could employ to extract natural products, effect of solvents in these extraction methods, and ultimately the estimation of various compounds in the extract. In this context, we were interested in studying the extraction of hemp seeds to estimate the amount of $\Delta^9$-THC, and if the extraction method could influence the estimation in commercial hemp seed. In this study, we report the extractions and analyses of three food-grade hemp seeds, the potential for underestimation of the controlled substance $\Delta^9$-THC, and the variability one might encounter due to the differences in extraction efficiencies, and discuss the bearing of these results onto public safety.

## Materials and Methods

### Materials

Three brands (brand# 1, 2 and 3) of hemp seeds were purchased from local supermarkets in Toronto, Canada, and were used as such in the laboratory experiments. All experiments, including extractions and analyses, were conducted under the appropriate Controlled Drugs Substances Dealer License granted to University Health Network. For ultra performance liquid chromatography (UPLC) analysis, HPLC-grade methanol and MilliQ® water were used for the preparation of the eluents. A Biotage® Initiator microwave was employed for all microwave-related experiments. Sample solutions were analyzed on a Waters® ACQUITY UPLC H-Class System equipped with Quaternary Solvent Manager, Sample Manager FTN, and Acquity UPLC® BEH column (2.1 × 50 mm, C18, 1.7 $\mu$m). A Waters MS 3100 mass spectrometer was used to monitor the samples in both the positive (ES+) and negative (ES–) modes. The injection plate and column were maintained at 15°C and 40°C, respectively. Cerilliant® standards for $\Delta^9$-THC, $\Delta^9$-tetrahydrocannabinolic acid ($\Delta^9$-THCA), cannabidiolic acid (CBDA), and CBD were purchased from Sigma-Aldrich® as Certified Reference Standards in the form of 1.0 mg/mL solutions in methanol or acetonitrile.

### Extraction

Four extraction methods were used to extract resins from three brands of food-grade hemp seeds. Each brand of hemp seeds was subjected to each extraction procedure thrice to assess any variability that might arise from the extraction procedure itself. Yields of resin obtained are based on the reweighed seeds.

1. Microwave extraction. Hemp seeds (1 g) were macerated in a mortar using a pestle, reweighed and then transferred into a vial, and suspended in ethanol (10 mL). The vial was sealed and the suspension was heated in a microwave to 150°C with stirring at 900 rpm for 20 min. The suspension was allowed to cool to room temperature and filtered on a pad of Celite® (2 g) and activated carbon (0.25 g). Solids were washed with additional solvent, and all fractions were concentrated to dryness under reduced pressure at 25°C to obtain a sticky resin (yield: 27–38%).
2. Sonication. Hemp seeds (1 g) were macerated, reweighed, and then transferred to a beaker. The macerated seeds were suspended in ethanol (26 mL), and the suspension was sonicated for 20 min after which the solvent was decanted. The sonication was repeated two additional times, collecting the solvent by decantation, refilling with an equivalent amount of solvent, and a

10-min break between each sonication session. All decanted solvent fractions were combined and filtered on a pad of Celite (1 g) and activated carbon (0.25 g). The solids were washed with additional solvent and concentrated to dryness under reduced pressure at 25°C to obtain a sticky resin (yield: 23–40%).

3. Soxhlet extraction. Hemp seeds (2 or 3 g) were macerated with a mortar and pestle, reweighed, and transferred into a cellulose extraction thimble (43 × 123 mm; 2 mm thickness). The thimble was placed in a Soxhlet extractor (size: 55/50), and ethanol (350 mL) was added to the extractor and refluxed for 4 h. Crude extract was then cooled to rt, and concentrated to dryness under reduced pressure at 25°C to obtain an oily resin (yield: 24–38%).
4. Supercritical fluid extraction (SFE). Hemp seeds (1 or 2 g) were macerated with a mortar and pestle, reweighed, and transferred to an extraction vessel. The extraction was performed using supercritical $CO_2$ as solvent A and ethanol as solvent B. The photodiode array detector was used to monitor the extract, with the range set to 200–600 nm. The back-pressure regulator was set to 12 MPa for the SFE, and other conditions include the following: flow rate = 10 mL/min for both $CO_2$ and slave pumps, and 1 mL/min for the make-up pump; temperature = 40°C; and gradient: 0–25 min: solvent A, 100% → 50%, and solvent B, 0% → 50%; 25–26 min: solvent B, 100%; and 26–30 min: solvent A, 100%. The acquisition time was 30 min and the total run time was 30.2 min. All fractions were combined and concentrated to dryness under reduced pressure at 25°C to obtain the extract as a resin (yield: 31–37%).

Extracts in the form of concentrated resins were used as such for the analysis and quantification of cannabinoids. A 10 mg/mL stock solution of the resin was prepared with a 70:30 methanol:water solution with 0.1% formic acid. A 100 $\mu$L aliquot of the stock solution was then diluted with 100 $\mu$L of mobile phase (70% MeOH in water, with 0.1% formic acid) and filtered to obtain a 5 mg/mL sample solution for analysis.

### Analysis

Sample injection volume was 10 $\mu$L, at a mobile phase flow rate of 0.6 mL/min for a total run time of 6 min. Two mobile phases, water/0.1% formic acid (phase A), and methanol/0.1% formic acid (phase B), were used and gradient conditions were used for elution: 0–4.5 min: 30% → 0% phase A and 70% → 100% phase B, 4.5 → 5 min: 100% phase B, and 5 → 6 min: 30% phase A and 70% phase B. Internal standard was benzophenone (10 $\mu$g/mL solution in MeOH), and each sample was spiked with 9.6 $\mu$L of internal standard before analysis. Each sample was analyzed in triplicate.

### Quantification

Chromatograms were obtained from the 315 ES+ and 357 ES– single ion recordings (SIRs). Signals on the chromatograms at retention times of 2.73 min ($\Delta^9$-THC) and 1.83 min (CBD) in the ES+ mode as well as 3.48 min ($\Delta^9$-THCA) and 1.95 min (CBDA) in the ES– mode were integrated to determine the areas-under-the-curves (AUCs) for each phytocannabinoid. In addition, AUC of the internal standard was obtained from the signal at 0.55 min in the 183 ES+ SIR and used in the analyses.

### Interpretation

All extracts were analyzed for the concentrations of $\Delta^9$-THC, $\Delta^9$-THCA, CBDA, and CBD. Thus, concentration standard curves for $\Delta^9$-THC, CBD, $\Delta^9$-THCA, and CBDA were generated using the respective cannabinoid standards of various concentrations and internal standard (Supplementary Fig. S1). These standard curves were used to estimate the concentrations of the above analytes in the extracts. Lower limits of detection for $\Delta^9$-THC, $\Delta^9$-THCA, CBD, and CBDA are 1.0, 1.0, 2.5, and 1.0 ng/mL, respectively, and the lower limits of quantitation are 2.5, 2.5, 5.0, and 2.5 ng/mL, respectively.

## Results and Discussion

Commercial hemp seeds are marketed for their high nutritional values, but due to their relationship to *Cannabis* spp. of plants, there is a potential for the presence of phytocannabinoids in these seeds. By regulation, total amount of $\Delta^9$-THC (whether in its acid form, $\Delta^9$-THCA, or as neutral $\Delta^9$-THC) must be less than 10 $\mu$g/g of hemp seeds (10 ppm) in Canada, and similar regulations exist in other countries where hemp seeds are legal. Hemp seeds from three brands in local supermarkets were purchased and brought to the laboratory. Each brand of hemp seeds was subjected to four different extraction protocols, and each protocol was repeated thrice to account for any variability due to the extraction procedures and associated errors. In total, 36 extracts were obtained

from the three brands and analyzed using UPLC-mass spectrometry to quantify the two major phytocannabinoids, $\Delta^9$-THC and CBD. We expected the quantity of $\Delta^9$-THC to be within the regulation limits and CBD to be in relatively higher quantities, as one would expect in hemp seeds. As it is common in the *Cannabis* spp. plants, majority of phytocannabinoids such as $\Delta^9$-THC and CBD exist in their carboxylic acid precursor forms, $\Delta^9$-THCA and CBDA (Fig. 1). Subjecting the extract or resin to high degree of temperature converts these acid precursors into decarboxylated forms, $\Delta^9$-THC and CBD. However, we calculated the total $\Delta^9$-THC equivalency (including $\Delta^9$-THCA and $\Delta^9$-THC found in each extract) to assess the total concentrations; similar procedure was used for the total concentration of CBD.

Extraction methods employed in this investigation utilize somewhat different principles to extract the phytocannabinoids from the hemp seeds into the solvent. Microwave-based extraction method used ethanol as the solvent, but at temperatures up to 150°C with stirring; majority of the acid forms, $\Delta^9$-THCA and CBDA, would be converted into the corresponding neutral forms, $\Delta^9$-THC and CBD, due to exposure to high temperature. This extraction process is also expected to offer high solubility to the phytocannabinoids due to heating to higher temperatures. Sonication was conducted at an ambient temperature using ethanol as the solvent, and is expected to help release compounds from the plant materials. SFE was conducted using a mixture of supercritical $CO_2$ and ethanol as solvent, at high pressures, but temperature was maintained at 40°C; thus, the extraction efficiency depended on the solubility of phytocannabinoids in supercritical $CO_2$ and ethanol mixture. Most exhaustive extraction, due to high temperature and long extraction time, is likely to be Soxhlet extraction, which was performed at the reflux temperatures in ethanol and for up to 4 h. Among these four methods, one would anticipate the highest yield of phytocannabinoids from Soxhlet extraction. Since ethanol was used in all these extraction methods, differences in extracted quantities of phytocannabinoids can be attributed to the extraction methods themselves.

The concentrations of $\Delta^9$-THC, $\Delta^9$-THCA, CBD, and CBDA, along with total $\Delta^9$-THC (i.e., $\Delta^9$-THC + $\Delta^9$-THCA) and total CBD (CBDA + CBD) from each brand of hemp seeds, using each of the four extraction procedures, are shown in Table 1, and are plotted in Figure 2. The discussion and interpretations henceforth are in the context of total $\Delta^9$-THC and total CBD.

We observed large standard deviations associated with each extraction of the same brand of seeds. Each extraction was performed thrice to be able to assess the experimental variability during extraction, and based on this large standard deviation, it appears that the extracts could show reasonable variability in the assessed phytocannabinoids, and this deviation may also be due to the nonhomogenous hemp seed bulk material. Either way, these variations warrant the analysis of multiple samples of hemp seed from different parts of the bulk material to assess total amount of phytocannabinoids, as accurately as possible. For brand# 1, all four extraction methods yielded approximately similar phytocannabinoids concentrations, that is, total $\Delta^9$-THC and CBD (Fig. 2A). Total CBD concentration ranged from $217 \pm 102$ to $227 \pm 111$ $\mu$g/g, and all four methods of extraction viz. microwave-based extraction, sonication, SFE, and Soxhlet extraction yielded similar results. Total CBD is expected to be relatively higher in concentration in hemp seeds and is reflected in these measurements. Total $\Delta^9$-THC has shown some variation based on the extraction method: sonication and Soxhlet extractions showed the amount of total $\Delta^9$-THC to be $70 \pm 26$ and $79 \pm 32$ $\mu$g/g, whereas microwave and SFE extracts showed $115 \pm 55$ and $126 \pm 57$ $\mu$g/g, respectively (Table 1). At the outset, all four quantities are several fold higher than the regulatory limits on $\Delta^9$-THC

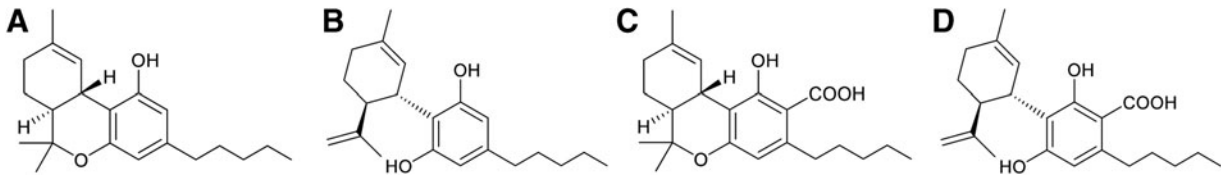


**FIG. 1.** Chemical structures of **(A)** $\Delta^9$-THC, **(B)** CBD, **(C)** $\Delta^9$-THCA, and **(D)** CBDA. THCA, tetrahydrocannabinolic acid.

**Table 1. Estimated Concentrations of $\Delta^9$-Tetrahydrocannabinol and Cannabidiol in the Hemp Seeds (in $\mu$g/g of Hemp Seeds)**

| Brand# | Extraction method | $\Delta^9$-THC | $\Delta^9$-THCA | Total $\Delta^9$-THC | CBD | CBDA | Total CBD |
|---|---|---|---|---|---|---|---|
| 1 | Microwave | 95±44 | 20±11 | 115±55 | 224±109 | 3±3 | 227±111 |
| | Sonication | 54±14 | 16±12 | 70±26 | 27±9 | 197±44 | 224±51 |
| | Soxhlet | 66±28 | 13±4 | 79±32 | 60±34 | 157±68 | 217±102 |
| | SFE | 97±33 | 29±24 | 126±57 | 49±13 | 174±93 | 223±106 |
| 2 | Microwave | 16±13 | 1±1 | 17±14 | 2±4 | 1±0 | 3±4 |
| | Sonication | 63±96 | 5±5 | 68±101 | 18±27 | 71±99 | 89±126 |
| | Soxhlet | 37±5 | 17±8 | 54±13 | 16±2 | 69±19 | 85±21 |
| | SFE | 63±7 | 12±5 | 75±12 | 13±4 | 159±27 | 172±31 |
| 3 | Microwave | 10±4 | 1±0 | 11±4 | 6±9 | 1±0 | 7±9 |
| | Sonication | 13±5 | 2±1 | 15±6 | 8±6 | 12±8 | 20±14 |
| | Soxhlet | 44±7 | 47±21 | 91±28 | 54±36 | 36±15 | 90±51 |
| | SFE | 19±3 | 4±1 | 23±4 | 9±7 | 13±2 | 21±9 |

Total THC and total CBD are the total observed weights of THC and THCA, and CBD and CBDA.
CBD, cannabidiol; CBDA, cannabidiolic acid; THC, tetrahydrocannabinol; THCA, tetrahydrocannabinolic acid.

quantities in hemp seeds in Canada (red line in Fig. 2A), and depending on the method employed for extraction, the estimation of $\Delta^9$-THC would be 7- to 12-fold higher than the legal limit (10 $\mu$g/g of hemp seeds in Canada).

Extractions of brand# 2 hemp seeds exhibited more variance, where total $\Delta^9$-THC amounts were estimated to be 68±101, 54±13, and 75±12 $\mu$g/g of hemp seeds using sonication, Soxhlet, and SFE extractions respectively, all of which are fivefold to sevenfold higher than the permitted limit (Fig. 2B), whereas microwave extraction estimated the total $\Delta^9$-THC content to be 17±14 $\mu$g/g only. Variations on the CBD estimates are even more significant, where the variation ranged from 3±4 $\mu$g/g (using microwave technology) to 172±31 $\mu$g/g (SFE) of hemp seeds. It is interesting to note that a different brand led to a completely different profile in the phytocannabinoid variations (brand# 1 vs. 2), and the results based on the extraction method employed are different as well.

For brand# 3, three extraction methods concurred with the estimation of the phytocannabinoids, viz. microwave extraction, sonication, and SFE estimated the CBD in the rage of 7±9 $\mu$g/g to 21±9 $\mu$g/g, and total $\Delta^9$-THC content in the range of 11±4 to 23±4 $\mu$g/g hemp seeds (Fig. 2C). However, Soxhlet extraction indicated that the amount of CBD and total $\Delta^9$-THC in brand# 3 hemp seeds to be 90±51 and 91±28 $\mu$g/g of hemp seeds, respectively. While the former estimations indicate that total $\Delta^9$-THC is closer to the legal limit in hemp seeds, the latter method indicated it to be up to nine folds higher than the legal limit, and this is a significant difference. Overall, none of the brands using any of the methods could convincingly be confirmed that the total $\Delta^9$-THC content is within the legal limits of 10 $\mu$g/g of hemp seeds. It is also noted that the phytocannabinoid content exhibited a significant variation even among batches from the same brand, reflecting both the inhomogeneous nature of seeds as well as the variations in quantification based on the extraction process.

According to Health Canada's Industrial Hemp Technical Manual, the current approved procedure of $\Delta^9$-THC quantification in hemp involves the sonication of 3 g of dried leaf powder in hexanes followed by analysis by gas chromatography.[11] There is no mention of testing procedures for any other parts of the hemp plant, including its seeds. Using a similar hexane-sonication procedure, quantification conducted by Ross et al., obtained $\Delta^9$-THC concentrations of 0–12 $\mu$g/g for fiber-type cannabis seeds.[7] In this study, ethanolic extraction using sonication exhibited significant variation from 17% to 92% of the maximum yield across the three brands of hemp seeds. This inconsistency could be attributed to the higher oil content within hemp seeds compared to the rest of plant. Due to hydrophobicity of the $\Delta^9$-THC molecule, it is expected to partition more strongly into the seed material, leading to the gross underestimation of $\Delta^9$-THC content by sonication.

$\Delta^9$-THC is a nonselective partial agonist of the CB1 and CB2 receptors, and elicits a variety of physiological effects, including analgesia, appetite stimulation, motor neuron inhibition, and CNS sedation, when bound to CB1.[12] $\Delta^9$-THC is highly potent and has a $K_i$ <50 nM for both CB1 and CB2 in humans.[13] In a study involving adult males who were infrequent users of cannabis, a 15 mg oral dose of THC was found to impair episodic memory and increase task error rates, 2 h after its administration.[14] Based on the results obtained in this study, 120 g of hemp seeds from brand# 1 could

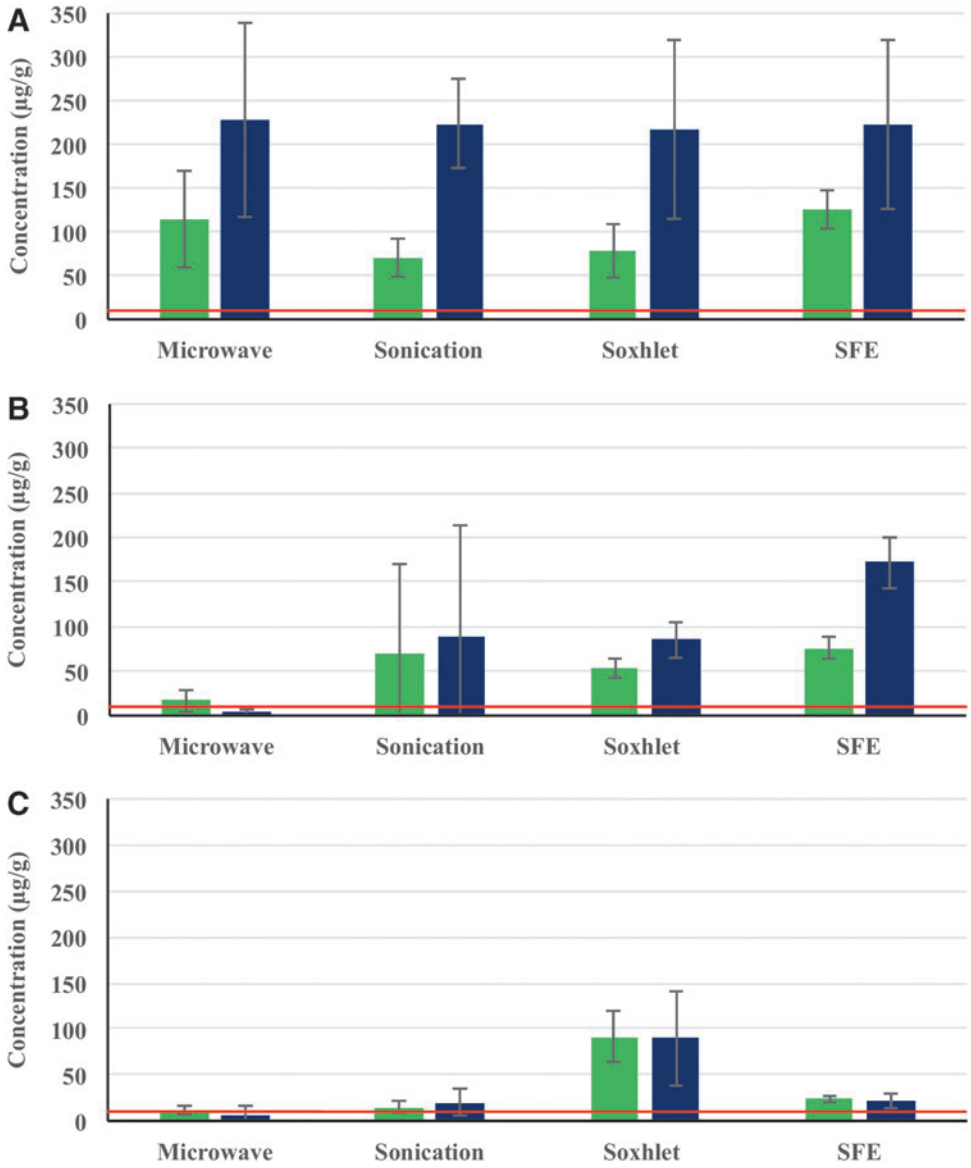


**FIG. 2.** Total $\Delta^9$-THC (green bars) and CBD (blue bars) content ($\mu$g/g of hemp seed) in the consumer-grade hemp seeds, in brand# 1 **(A)**, brand# 2 **(B),** and brand# 3 **(C)**. Legal limit of $\Delta^9$-THC per gram of hemp seeds (as per Health Canada) is shown as a horizontal red line. CBD, cannabidiol; THC, tetrahydrocannabinol.

contain an equivalent quantity of $15 \pm 3$ mg of total $\Delta^9$-THC, using the quantity estimates from SFE. Suggested serving size for an adult for most consumer brands of hemp seeds is 30 g, and this is equivalent to $3.8 \pm 0.6$ mg of total $\Delta^9$-THC, when using brand# 1 hemp seeds. It is also noted that a significant portion of the total $\Delta^9$-THC content exists in the form of the acid precursor $\Delta^9$-THCA, which is not known to exhibit psychoactivity.[15] However, exposure to heat (due to cooking or other reasons) could always generate $\Delta^9$-THC. However, in the absence of strong heating, the seeds' effective $\Delta^9$-THC concentration is expected to be lower than their total $\Delta^9$-THC content, lowering the risk of acute phytocannabinoid poisoning from direct consumption. Chinello et al. reported a case of subacute poisoning from the sustained consumption of a relatively $\Delta^9$-THC-poor product by a toddler.[9] Such subacute poisoning is always a possibility when hemp

seeds carry higher quantities, such as 10- and 12-fold higher than the recommended limits, or the concentrations of $\Delta^9$-THC are not estimated accurately.

In an earlier study, Ross et al. conducted an investigation to determine $\Delta^9$-THC content in drug- and fiber-type (hemp) cannabis seeds.[7] Hemp seeds in this study were found to contain 0–12 $\mu$g $\Delta^9$-THC per 1 g of seeds, but $\Delta^9$-THC in drug-type cannabis seeds was in much higher levels (35.6–124 $\mu$g/g). It was found that majority of $\Delta^9$-THC was located on the surface of the seeds, and a wash with chloroform removed upto 90% of $\Delta^9$-THC. It was suggested that fluctuations in the $\Delta^9$-THC content of different replicates of the same type of seeds could be the result of the degree of contamination on the outside of the seeds. In this study of consumer-grade hemp seeds acquired from the grocery stores, highly variable, but above the legal limit of, $\Delta^9$-THC may suggest either contamination by drug-type cannabis seeds or improper washing of the seeds.

$\Delta^9$-THC primarily undergoes liver metabolism through CYP3A4 and CYP2C9.[16] Due to the polymorphic nature of P450 enzymes,[17,18] people consuming hemp seeds may gradually accumulate $\Delta^9$-THC due to its slow metabolism or relatively long half-life in the body, leading to potentially higher concentrations. In the report by Chinello et al., $\Delta^9$-THC concentration in the prescribed hemp seed oil was 0.06%, that is, 0.6 mg of total $\Delta^9$-THC in 1 g of hemp seed oil, and the child was administered two teaspoons ($\sim$10 mL or 9.2 g) a day for 3 weeks before the incidence of neurological symptoms.[19] This amounts to 5.52 mg total $\Delta^9$-THC per day, when one consumes 10 mL above hemp seed oil. If one were to compare these total $\Delta^9$-THC levels, a similar quantity of total $\Delta^9$-THC (5.52 mg) is contained in $\sim$44.2 g of hemp seeds (brand# 1, total $\Delta^9$-THC estimate based on SFE extraction), and this is certainly a normal quantity that consumers may consume as part of their daily food consumption. In people with liver impairment or patients consuming other drugs such as ketoconazole (an inhibitor of CYP3A4) or sulfaphenazole (an inhibitor of CYP2C9), one would expect the metabolism of $\Delta^9$-THC to be slower, and would be at risk for adverse effects upon the consumption of hemp seeds with higher concentrations of total $\Delta^9$-THC.[16,20,21] However, we note that the bioavailability of $\Delta^9$-THC is only 10−20% and could vary if consumed along with fatty food, and such factors would influence the plasma levels of $\Delta^9$-THC.[22–24]

The other major phytocannabinoid in hemp, CBD, is an antagonist of CB1 and CB2 with relatively weak binding affinities.[12] While CBD is not known to exhibit psychoactive properties, CBD can be cyclized into $\Delta^9$-THC when incubated with artificial gastric juice at 37°C.[25] Given that CBD was present in generally higher amounts than $\Delta^9$-THC, the conversion of CBD into $\Delta^9$-THC in the stomach after consumption may further contribute to the psychoactivity of hemp seeds.

## Conclusion

In comparison, Soxhlet extraction provided consistently higher yields of $\Delta^9$-THC, although it takes longer time than other methods for extraction. This suggests the importance of heating and prolonged solvent cycling in extracting phytocannabinoids from lipid-rich materials such as hemp seeds. $\Delta^9$-THC concentrations of up to 125 $\mu$g/g of hemp seed were found in food-grade hemp seeds, and all evaluated brands contained higher amounts than the legal threshold of 10 $\mu$g $\Delta^9$-THC per gram of hemp seeds. Exposure to higher amounts of $\Delta^9$-THC may cause neurological symptoms especially for poor metabolizers of cannabinoids. It would be presumptuous to conclude the source of this excessive $\Delta^9$-THC in the consumer-grade hemp seeds, but could be either contamination during harvesting/processing of the seeds or higher levels of biosynthesis, which is unlikely. Current methods for validating $\Delta^9$-THC content in hemp may be providing lower and/or inconsistent yields for hemp seeds and could lead to the underestimation of $\Delta^9$-THC content. A more robust extraction methodology such as Soxhlet extraction may be more appropriate for the testing of hemp seed products. One may also consider employing washing of hemp seeds with ethanol or other similar solvents, to remove any contamination to the seeds before packaging; but such change from current practice and new processes must be thoroughly investigated before implementation for consumer marketing. Based on the above findings, it is also recommended that the hemp seeds be analyzed specifically for phytocannabinoid content before release into consumer markets.

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L.P.K. and H.A.C. serve on the scientific and medical advisory board of Scientus Pharma, Inc. and receive a consulting fee.

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### Abbreviations Used

AUC = area-under-the-curve
CBD = cannabidiol
CBDA = cannabidiolic acid
IHR = Industrial Hemp Regulations
SFE = supercritical fluid extraction
SIR = single ion recording
UPLC = ultra performance liquid chromatography
$\Delta^9$-THC = $\Delta^9$-tetrahydrocannabinol
$\Delta^9$-THCA = $\Delta^9$-tetrahydrocannabinolic acid