Sampling Cannabis for Analytical Purposes
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Overview

This paper discusses the practice and regulatory implications of sampling cannabis for potency and purity tests. It proceeds in three parts: first, by discussing proper procedures by which a small, representative test sample can be taken from a larger lot of cannabis; second, by discussing the natural levels of heterogeneity in the cannabis plant; and finally, by discussing the cost burdens of different sampling regulations, including the size of a lot.

Initiative 502 established a program for chemically testing regulated cannabis, in order to protect consumers from unhealthy product and inform them of a product’s potency and purity. Such a program will require a policy on sampling methodology. Sampling is an integral aspect of cannabis testing, and if done dishonestly or improperly, it may skew the results of an otherwise reliable testing process. Of particular regulatory importance is to prohibit producers or testers from manipulating sampling procedures in order to exaggerate a product’s reported potency or purity, and consequent retail value. Another important decision is the appropriate size of the sampling lot. Both of these policy decisions are important in order to establish a high standard for industry practice and to prevent intentional manipulation of results.

In the regulations proposed by the WSLCB on July 3rd, 2013, the unit of usable cannabis from which a sample is pulled is referred to as a lot. A lot of flower must come from one or more plants of the same strain and weigh no more than five pounds[WAC 314-55-010(9)]. The unit of extract or infused cannabis product from which a testing sample is pulled is referred to as a “batch.” A grower’s yield or harvest, typically pulled from a set of plants of the same strain grown under the same conditions, is broken up into lots, and those lots are submitted for testing. It can be assumed that growers will create lots as large as they are allowed, and that they will want to lose as little product to testing as possible and minimize their testing costs.

In determining a requisite sampling lot size, the Washington State Liquor Control Board (WSLCB) faces an inherent trade-off between accuracy (or representativeness) in testing results and regulatory cost. On one hand, a larger lot size eases the burden on the cannabis industry by requiring fewer tests, since each lot must be individually divided into a sample and run through the required tests. Moreover, since sampled material cannot be sold, a larger lot size decreases the dead loss of unsellable cannabis. On the other hand, if there is a large amount of variation within an individual lot, a sample from within that lot might have drastically different properties than another part of that lot. This is the problem introduced by the heterogeneity of cannabis, in part because grinding the product into a homogenous mixture decreases its retail value, and in part because of the biological properties of the plant.

Cannabis plants exhibit heterogeneity in two regards: across different parts within the same plant and across different plants within the same strain. Cannabis plants have been subject to decades (if not centuries) of intense domestication, both through breeding and cloning, creating a wide variety of strains each with their own biological peculiarities. Through a combination of conventional wisdom among growers and scientific studies, we
know that some strains can be cloned with higher levels of similarity than others. This characteristic would reduce the level of variety from one plant to another, provided both are members of the same strain. Another type of heterogeneity, intra-plant, is important to sampling procedures. This paper will review the mixed literature on levels of heterogeneity in cannabis plants and identify policies appropriate to deal with those levels of natural heterogeneity.

As the I-502 market develops, and more growers demonstrate their capacities to produce and reproduce strains with consistent cannabinoid profiles, the WSLCB may consider developing a varietal registry of different cannabis strains. Such a registry could establish expected potency levels and variances for particular strains. This information could be used both to verify the accuracy of a particular test result and to distinguish those varieties with the most severe levels of variance. A possible cost-saving measure would be to allow larger lot sizes or more relaxed testing regulations for those strains known to exhibit lower levels of variation. Such research could also facilitate the distinction between one strain and another, as defined in WAC 34-55-102(10) of the CR-102 for cannabis producer licenses and requirements.

This paper makes two assumptions about the procedures of testing laboratories. First, we assume a high degree of competence from laboratories, and of the accuracy, robustness, and reproducibility of their methodologies. It is imperative that any laboratory providing testing be able to demonstrate at least 95% accuracy of the testing methodology by passing a blind proficiency test of random samples. Second, it is assumed that a chain-of-custody plan will be followed, such that no contamination will be introduced in the lab. Sterile handling in a biosafety hood (Class II, Type A bio-safety cabinet) is necessary for testing for microbiological contamination. Each facility should have a sample processing room and secure storage room. At the point of sample reception, a log should note the time of arrival, the recipient, the sender, and the lot and, when applicable, the batch number (USDA 2013).

Sampling of Raw Plant Material

Sampling
Sampling is the selection of a subset within a whole, in order to estimate characteristics of the whole. In the case of cannabis, this is harder than it may appear at first blush. First, cannabis naturally varies in chemical potency, both within a single plant and between one plant and another (and between strains); secondly, cannabis is commonly marketed as intact flower buds. For this reason, cannabis cannot be homogenized without permanently damaging the un-sampled product. Alcoholic beverages, for instance, do not face this second problem. Even if a company’s brewing or distilling process produces some vats with 6% alcohol and others with 7% alcohol, simply mixing the two vats together can standardize the product. Similarly, tobacco is generally baled, and cores are taken for quality analysis without damage to the bulk material. Performing the same procedure with cannabis would require grinding the entire crop into small bits, thereby reducing its aesthetic appeal and retail value. Since the heterogeneity in cannabis potency cannot easily be mixed away, this puts the onus on other ways for verifying that a sample is representative of its whole.
Since the psychoactive chemicals of cannabis are unevenly and non-randomly distributed throughout the plant, there exists an opportunity for producers to manipulate the sampling process in order to produce a sample that exaggerates their crops’ potency. THC content is commonly regarded to vary from the top to the bottom of the plant, or by the proximity to the light source. In the case of outdoor production, it is widely believed that flowers from the bottom of a plant receive less sunlight than those at the top of the plant; this is also purported to be true for indoor production, but the effect might be mitigated by carefully placing high-intensity lamps so that they shine more uniformly on all flowers in the plant. In either case, flowers that receive less exposure to light are likely to have lower cannabinoid and terpenoid content. Since a cannabis producer is typically aware which parts of the plant are most well lit, he often knows where to find the most potent flowers from the cannabis plant – typically, those at the top. Potentially, this represents a crucial information asymmetry between the producer and the testing agency. A producer may manipulate his crop’s potency ratings by deliberately selecting his plant’s most potent flowers and submitting them to the testing agency as representative of the entire plant’s (or crop’s) inflorescence.

There are several options to address this vulnerability, depending in part on whether samples are taken at the time of harvest or only after the harvest is dried. If samples are taken at the time of harvest, cannabis should be gathered in groups according to their exposure to light. This could be achieved by adhering to height standards (such as one sample taken at x feet and another at y feet) or distance in lumens away from the light source. These samples would need to be cured or dried prior to analysis. In this case, a trained field inspector (as recommended by the USDA-Animal and Plant Inspection Service Plant Protection and Quarantine APHIS-PPQ) could sample at the time of harvest, selecting flowering tops taken from different parts of the plant. Health Canada has prescribed a procedure for industrial hemp that could be adapted to this purpose (Canada 2008). If plants were trellised, then a height variable would not be necessary. Each plant to be sampled needs to be readily accessible from all sides of the plant, and in its original growing location. Official samples should be brought to the testing location by the inspector.

Another option is to allow producers to lot cannabis according to their own methods, but then have testing agencies select a random sample from within those lots. In this case, growers would first dry and lot their own harvests. The agencies would then randomly sample the lots using established methodologies. In this scenario, growers might choose to lot their harvest based on flower size, light exposure, or other strategic considerations. If a lot is smaller than two kg or under the five lb. lot definition, then whatever is 20% of the lot can be used for sampling, as long as the final sample taken for cannabinoid analysis is at least 2.5 g. The rest of the plant material can be returned to the grower (except for additional material needed for microbiological testing) when performed on a separate sample. Allowing growers to perform their own semi-quantitative testing at this level could represent a cost savings to the grower. A semi-quantitative methodology might be high performance thin layer chromatography or infrared technology.

In either case, it is important that growers cannot knowingly provide testing agencies with samples that are unrepresentative of the lot. In the first case, this is accomplished by
preventing growers from being able to select the sample; in the second case, this is accomplished by sampling from a larger lot than is normal. Regardless of the sampling protocol, any laboratory or method used must demonstrate precision (Figure 1), intra-assay accuracy (Figure 2), and reproducibility over time (Figure 3). These data were generated and compiled using cannabis samples in California as part of an internal single-lab validation methodology by Integrated Analytical Systems, a bio-analytical company in Berkeley, California.

Figure 1: Method Precision
Figure 2: Method Accuracy

One Sample Analyzed 5 Times

<table>
<thead>
<tr>
<th>Analysis 1</th>
<th>Analysis 2</th>
<th>Analysis 3</th>
<th>Analysis 4</th>
<th>Analysis 5</th>
<th>Relative Variability</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.81%</td>
<td>8.06%</td>
<td>8.02%</td>
<td>7.88%</td>
<td>7.91%</td>
<td>±2.3%</td>
</tr>
</tbody>
</table>

Figure 3: Method Reproducibility Over Time

Lot of Flowers (1 lb bag)

5 Separate Samples from Same Lot (2 gm)

<table>
<thead>
<tr>
<th>Day of Analysis</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Sample 4</th>
<th>Sample 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>9.11% THC</td>
<td>8.42% THC</td>
<td>8.62% THC</td>
<td>8.01% THC</td>
<td>8.56% THC</td>
</tr>
<tr>
<td>Day 60</td>
<td>9.03% THC</td>
<td>8.62% THC</td>
<td>8.40% THC</td>
<td>7.73% THC</td>
<td>8.69% THC</td>
</tr>
<tr>
<td>Difference</td>
<td>0.5%</td>
<td>2.4%</td>
<td>2.7%</td>
<td>3.5%</td>
<td>1.5%</td>
</tr>
</tbody>
</table>
Sample preparation for useable cannabis

Established methodologies exist for preparing a sample of useable cannabis for testing. These methods vary slightly based on the intent of the test (e.g. detecting pesticides, or potency, or microbiotics). However, for the most part a simple and common protocol should be used for this process.

Selecting the sample

Cannabis inflorescence (fruiting tops or flowers) or “trim” is sampled when performing testing for potency and/or microbiotics. The “fan leaves” of the plant are used for pesticide testing. Broad leaf should be collected from each plant in the lot. This sampling could be done at another time prior to harvest.

A test specimen will be comprised of inflorescence taken from a lot of plants, and a representative sample of 10 grams per kilogram (or 2% of the total lot) or trim from the flowers (10 grams per kilogram or 2%). A “lot” of plants can be distinguished by count, by lumens, or defined by the space receiving approximately the same conditions with regard to light, moisture, nutrition, CO₂ and temperature recommended to be 20 plants (Mechtler et al. 2004).

Homogenization of the raw sample

The plant sample must be made homogeneous for test results to be representative. Homogenization requires the sample be broken down to a form that can be mixed effectively, comparable to the process of turning wheat into flour.

First, the sample should be ground to a size of around 0.5 cm in size and thoroughly mixed. There is some disagreement about grinding a sample because trichomes can be lost in the process, but without grinding a sample cannot be as homogeneous. To minimize the leaching of resins, grinders made of silanized glass or stainless steel are recommended over wood and plastic. Regardless of the material, every element of the grinder must be thoroughly cleaned with solvent rinses between samples.

Once grinding is completed, the next step is quartering. Quartering ensures that every part of the sample is sufficiently mixed to have an equal chance of being selected for testing. The ground sample is gathered into an even and square-shaped heap. Next, it is divided diagonally into four equal parts. The two opposite parts are then taken and carefully mixed. This portion is now placed in another square shape and divided diagonally. Two opposite parts are taken and carefully mixed. This 2.5-gram sample can be used for the analysis of cannabinoids, terpenoids, or other phytochemicals.

The remainder of the sample can be used for microbiological testing. Sampling methods could be further refined and validated through a series of experiments that the WSLCB should conduct to determine the relative variance of different sampling methodologies for cannabis crops. This data can then be made available to the entire industry, and may serve to refine the regulatory process.
Obtaining a representative sample for analysis

A two-gram sample of flower or trim should allow for a confidence of approximately 12% relative variability (or five grams for a relative error of approximately 5%; see Table 1 and Figure 4). In developing the laboratory protocol, a five-gram representative sample is needed for the least variability. However, as the data below reflect, 2.5 grams would allow for an acceptable variation across a single sample. Analytical performance standards for hemp are described in Table 2 (Canada 2008).

Table 1

<table>
<thead>
<tr>
<th>Sample Weight</th>
<th>Variability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 gram</td>
<td>±9.9%</td>
</tr>
<tr>
<td>2 grams*</td>
<td>±5.7%</td>
</tr>
<tr>
<td>3 grams</td>
<td>±4.3%</td>
</tr>
<tr>
<td>5 grams</td>
<td>±1.5%</td>
</tr>
</tbody>
</table>

* recommended weight to submit for testing

Figure 4

Graphical Representation of Variability

Table 2: Analytical performance standards for hemp, and parameters for THC analysis: the limit of detection (LOD), limit of quantification (LOQ), and acceptable linear range for reference standards. These values may be adopted and required of labs that want to be certified to test cannabis in Washington State.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Concentration of THC in industrial help, other than its derivatives or products containing those derivatives</th>
<th>Concentration of THC in derivatives of industrial hemp, or products containing those derivatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum limit of detection</td>
<td>0.1% (w/w)</td>
<td>4.0 μg/g</td>
</tr>
<tr>
<td>----------------------------</td>
<td>------------</td>
<td>---------</td>
</tr>
<tr>
<td>Minimum limit of quantification</td>
<td>0.1% (w/w)</td>
<td>4.0 μg/g</td>
</tr>
<tr>
<td>Intra-assay precision</td>
<td>C.V. (coefficient of variation) ≤ 10% at 0.3% (n=8)</td>
<td>C.V. (coefficient of variation) ≤ 10% at 10.0 μg/g (n=8)</td>
</tr>
<tr>
<td>Linear range</td>
<td>$r^2 \leq 0.98$ in the range of 0.1% to 1.0% (w/w)</td>
<td>$r^2 \leq 0.98$ in the range of 4.0 μg/g to 30.0 μg/g (w/w)</td>
</tr>
</tbody>
</table>

Sample preparation for extracts and cannabis-infused products

The global market for botanical and plant-derived drugs is expected to increase from $19.5 billion in 2008 to $32.9 billion in 2013. Finished products made from medicinal and aromatic plants are increasingly prescribed and bought over the counter. An extract is obtained as a solution by treating plants (or parts of them) with a solvent, which can then be further concentrated through evaporation, distillation, or some other process (WHO 2004). Liquids intended for oral consumption should be uniform, and finished products need to be handled in clean facilities and assayed for residual solvent and/or labeled with final ethanol or glycerol concentration. Mixed batches can be used for solvent extraction and a homogeneous sample needs to be submitted for final analytical determination of active ingredients. An herbal drug product may be a solid extract, a soft extract (partially evaporated), or a liquid extract (1:1).

Selecting the Sample

The sampling unit is a batch of extract (there may be more than one per lot) including tinctures and fatty oils of herbal materials. Extract lots are produced either by extraction, fractionation, purification, concentration, or other physical or biological processes. The final volume is the lot size, while “individual units” are the containers of product eventually sold from this lot. Extractions are preparations made by steeping or heating herbal materials in alcohol, glycerin and/or honey, or in other materials (WHO 2007). The size of the sampling unit should be scaled to be representative of the size of the lot of extract, and the sample size will determine an acceptable quality level. Resins and solid extracts should be sampled by weight, and liquids by volume.

Testing agencies should adequately homogenize each batch and take representative samples from three separate areas of the container (WHO 1998). The amount of the representative sample may be determined by the volume of the batch, again extracting a predetermined percent of the total volume. In the case of resinous material, it may need to be warmed on a heater/stirring device. This is now a “pooled” sample.
Homogenization of the pooled sample

Homogenization of the sample should occur by stirring or vortexing and may require heating of the sample. After homogenization, the sample should be quartered.

The process of quartering samples of a finished product is similar to quartering samples of dried flower. A sample is placed in a single container, and then divided into four equal volumes. Two parts are then combined and vortexed. This portion is now divided in half. Two opposite parts are taken and mixed. This representative sample can be used for the analytics of cannabinoids, terpenoids, or other phytochemicals.

The remainder of the sample can be used for microbiological or residuals testing. Sampling methods should be further refined and validated through a series of experiments that the WSLCB could conduct to determine the relative variance of different sampling methodologies for cannabis derived products. These data can then be made available to the entire industry, and provide guidance for the regulatory process.

Acceptance Sampling

Acceptance sampling could prove a viable alternative to the sampling methods described above. Acceptance sampling was originally applied by the U.S. military for the testing of bullets during World War II. If every bullet were to be tested in advance, no bullets would be left to ship. If, on the other hand, none were tested, malfunctions were likely to occur in the field of battle (Bheda 2010). Acceptable Quality Level (AQL) is a statistical measurement of the maximum number of defective goods considered acceptable in a particular sample size. If the AQL is not reached for a particular sampling of goods, manufacturers will review the various parameters in the production process to determine the areas causing the defects.

The AQL will vary from product to product. For example, medical products are more likely to have a more stringent AQL because defective products can result in serious health risks. Companies have to weigh the added cost associated with the stringent testing and potentially higher spoilage due to a lower defect acceptance with the potential cost of a product recall. Industry AQL charts could provide WSLCB with AQL protocol for botanical products made from cannabis extracts.

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The lot size, on the y-axis, is based on how many individual units will be on the market from a particular lot. Unit size is not defined by the chart, but by individual manufacturers. For instance, a CO₂ cartridge manufacturer’s unit would be a single cartridge, a baker’s unit may be an individual cookie, and a farmer’s unit might be a gram or an ounce. The AQL (the x axis) is the level of acceptance for the number of units that fall outside of quality parameters (how many). The numbers within the body of the chart are sample sizes. When working with a lot size of 2500 units at a 0.065% AQL, one would want a sample size of 200 units from each lot to ensure this level of confidence. As the AQL is reduced, confidence increases in the probability of units meeting quality parameters. Following our example, a 2500 unit lot with an AQL of 0.065% (with zero units falling out of specs in a sample of 200) has a statistical probability of producing two defective units. By contrast, a lot of 2500 units with an AQL of 1% (with zero units falling out of specs in a sample of 42) has a statistical probability of producing 25 defective units.
In order to implement this strategy for cannabis products, it would be necessary to convert what is currently a continuous variable (% concentration) to a discrete binary variable. Setting a threshold for acceptable quality or concentration of product would accomplish this.

Acceptance sampling protocol for the cannabis industry could take many forms. The practice is easily applied to products with obvious definitions of a unit, such as extracts or edibles because their contents have been homogenized. It will not be as clear how appropriate acceptance sampling could be for raw flower until more is understood about the heterogeneity of the crop. Theoretically a lot of flower would be broken up into predetermined units of sale and a certain number of those units selected for testing. Qualifications to deem a unit defective could be a certain level of contaminants or a cannabinoid profile that is too different from the goal for that strain.

**Varietal Registration and State-led Research Efforts**

For obvious historical reasons, the science and practice of cannabis cultivation and testing are not as well established or well documented as in other fields. Cannabis production and testing has historically been performed in secrecy, and private companies have been reluctant to construct and share large databases. This lack of shared knowledge constrains both the cannabis industry and regulators. Establishing an informational database or a center for research would deliver some benefit to the State of Washington, if it were to decide to spearhead such an effort.

One model of a shared informational database is a germplasm bank or a varietal registration, also known as a chemotaxonomic classification system and an associated seed repository, intended to illuminate the characteristics of different cannabis strains. Registration could occur upon a grower's demonstrated ability to reproduce a strain with a high degree of homogeneity. Such a strain could then, in effect, be trademarked without ownership rights and registered with the State as a “varietal.” This public database could be useful to both entrepreneurs in the private sector and regulators in the public sector. Over time, if a strain’s ability to retain phenotype is relatively strong, regulators may opt to relax testing requirements for a certain varietal, defraying testing costs in the long term. This registration could also help regulators to distinguish between different strains (“...a pure breed or hybrid variety of Cannabis reflecting similar or identical combinations of properties such as appearance, taste, color, smell, cannabinoid profile, and potency”). The issue of chemical fingerprinting is further discussed in the BOTEC paper, “1c. Testing for Psychoactives.”

More ambitiously, Washington could also opt to establish a research center within an existing state laboratory. Such a program could investigate many different aspects of the cannabis plant, including product consistency, identifying new varietals, fingerprinting cultivars, participating in the development of medically relevant strains, and determining whether there actually are ailment- or symptom-specific components. Such an effort could contribute to arguments to designate Washington as a “Protected Geographical Indication” or a “Protected Designation of Origin.” To date, such research has been blocked by federal regulations, although it is often taken for granted in other areas of agriculture.
However, Washington might pay all the costs of such an effort and reap only a small portion of the benefits. Although Washington, along with Colorado, has recently become a major player in the cannabis industry, this celebrity status might not last. Other states might legalize as soon as 2014 or 2016 – notably California – and they may do so with more business-friendly regulations, not to mention warmer and drier climates. Initiative 502 may have given Washington’s cannabis industry a head start, but other states will soon join the race. Leading a research effort might help Washington maintain that head start, but should not be expected to guarantee Washington’s spot as an industry leader in the long term.

### Heterogeneity

In this section we review studies concerned with the heterogeneity of cannabis across growing conditions and varietal strains. Cannabis is an inherently variable plant, with strong genetic and environmental contributions to variance in quality (Zamengo et al. 2013). In the interest of having products in the marketplace that are as predictable as possible (but still appropriately labeled), research and a list of needs should be required.

The contents of a lot should be as homogeneous as possible. Gathering lots using the criteria described—products of the same strain, flowers from consistent areas of the plant, similar bud size, etc.—relies largely on growing techniques that have been commonly accepted, but hardly corroborated by scientific protocol. This lack of information reveals vulnerability in sample testing. How effective could testing be if we cannot put forth an acceptable range of its representativeness? Under the regulations put forth by the WSLCB, a lot must consist of flower taken from plants of the same strain. However, there is little literature to tell us that plants of the same strain grown under identical conditions can be assumed homogenous, or to what extent they differ. To know how representative a sample is, we must know the extent of heterogeneity occurring in cannabis plants when grown in identical conditions.

There is a compelling need to learn much more about how growing conditions affect the phenotype and chemotype of the cannabis plant. To set forth a range of acceptable variation in a product, it is important to understand the extent of natural variation. Industry consensus is that the quality and quantity of light will affect THC production. Increasing lumens, particularly of specific wavelengths, may potentially increase THC production, but studies that suggest that cannabinoid content is more controlled by genetics (Fournier et al. 1987) than by other factors such as the quality or quantity of light a plant receives. Because cannabinoids are secondary metabolites (chemicals produced by the plant in response to stress), some suggest that light “stress” could increase their production. However, these secondary metabolites may have unknown roles and are not fully understood. There are many other variations in growing techniques, such as addition of CO₂ to the growing environment, nutrient mixtures, soil mixtures, humidity levels, pH balance of the soil, hydroponics, and water administration, that will dictate plant metabolism.

The genetic profile of cannabis has changed over the last several decades, as evidenced by analysis of cannabinoids in seized samples (Burgdorf et al. 2011; Mehmedic et al. 2010)
[See Figure 5]. This change in genetic stock is reflected in the relative changes in total THC content and also the ratio of THC to CBD.

Figure 5: Variability in THC/CBD in seized crops over time. Median, 25th and 75th percentiles of cannabis seized in California for 4,561 plants from 1998 to 2006.

### Typical Heterogeneity

Cannabis is an inherently variable plant, with strong genetic and environmental contributions to variance in quality factors. Given the interest in ensuring products in the marketplace are of known content and appropriately labeled, there is a need for research into the determinants of plant characteristics.

An analysis of hemp samples in Germany, Poland, France, and Hungary was undertaken to estimate the sample size needed for “routine control tasks” (Mechtler et al. 2004). One study found no association between plant size and THC content. They also found great consistency in hemp crops over years and consistent “intra-plant” levels of THC with as many as 30 samples from a single plant.

However, another study concluded that a varietally homogeneous hemp field might contain a significant number of plants behaving irregularly with respect to THC values. Further, the number of plants sampled for routine analysis was fixed by European Union (EU) regulations at 50 plants (regulation number (VO (EG) 1177/2000).

Few publications have explored this topic with regards to regulated indoor growth of cannabis. Growers often cultivate what is known as a genet: a “clonal colony” in which all of the individuals (ramets) have originated vegetatively from a single ancestor. One advantage of indoor production is an enhanced ability to carefully control soil nutrients and light, factors that can contribute greatly to variations in growth, biomass, morphology, and physiology of clones (Wang et al. 2012). Under these conditions, producers can provide
consistent treatment from one plant to another, and often to different parts of the same plant (for instance by the uniform position of lights). However, it is unknown what effect these conditions have on reducing the variance in plant chemotypes, or their psychoactive chemical content. For instance, two different plants cloned from the same “mother” might grow differently even if they are exposed to the exact same conditions. It may be in Washington State’s interest to commission or encourage such experiments, perhaps as part of a larger effort to form a varietal registry.

**Heterogeneity across strains**

Small and Beckstead (1973) were the first to survey cannabis accessions for cannabinoid variability. The University of Mississippi concluded that, phenotypically, cannabis might be a single species that has not stabilized and has many variations (Doorenbos et al. 1971). The researchers prepared fields and planted seed from several varieties, noting that environment and climate, not heredity, are the most important determinants of cannabinoid content. They also found a great deal of inter-plant variability in THC content, and report an interesting anecdote.

A cannabis plant alleged to have been grown in a closet with a tungsten light bulb was delivered to their facility. The authors described it as leggy, with greenish-yellow leaves, and yet the cannabis harvested analyzed for Δ⁹-THC at 6.8%, CBD: 0.26%, CBN: 0.28%. At this time, 6.8% THC was well above any of the outdoor plants with regard to THC production. In other words, this neglected specimen was remarkably successful at producing psychoactive cannabinoids, even though it may have been exposed to inferior soil and lighting conditions. This observation is instructive with regard to visible and ultraviolet lighting in indoor growing facilities. Clearly, a “stressed” plant produced a relatively greater amount of THC than any of the other varieties cultivated outdoors. While this report gave some information on heterogeneity across strains, unlike more contemporary farmers the researchers were not growing clones from a single plant.

In 2003, GW Pharmaceutical published a paper in *Genetics* which stated: “there is little doubt that environmental factors have a strong influence in modulating the amount of cannabinoids present in the different parts of the plants at different growth stages.” However, they report that cannabinoid profiles in general are under strong genetic control (the THC to CBD ratio, specifically) and that plants typically demonstrate high degrees of polymorphisms (or spontaneous genetic mutations) - up to 80% measured in fiber-type plants - which can account for variability (de Meijer et al. 2003). For plants that were double inbred clones (S₂'s: female lines with “pure fixed” chemotype), major cannabinoids ranged from between 84-98% of total cannabinoid fractions.

Figure 5: GW Pharmaceuticals shows how cannabinoid content is under genetic control and uses genetic manipulation to precisely control cannabinoid production.
Below, we compiled THC content data taken from the website of a Seattle-based medical cannabis facility. We randomly chose several strains: “Blue Dream” (n=20), “Blueberry” (n=8), “Jack Herer” (n=9) and “Harlequin” (n=9) for purposes of resale. Figure 6 shows THC concentration by weight, and summary statistics are given in Table 3. Assuming that the samples were tested accurately, these strains appear to have different rates of variability. The data, though very limited, suggest an approximate 25-30% variability in Blue Dream, 25% for Blueberry, 60% for Jack Herer, and 40% for Harlequin. (Due to a small sample size, these figures might not accurately represent characteristics of these strains in the larger market.)

Table 3: Statistics on Δ⁹-THC content for four commercial Cannabis varieties, from the Analytical 360 website, a medical cannabis laboratory in Seattle. The sampling and testing
methodology is unreported. Data in this table summarizes the data presented in Figure 6. These data do not necessarily represent typical characteristics of these strains.

<table>
<thead>
<tr>
<th></th>
<th>Blue Dream</th>
<th>Blueberry</th>
<th>Jack Herer</th>
<th>Harlequin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of observations</td>
<td>21</td>
<td>8</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Minimum</td>
<td>6.560</td>
<td>10.05</td>
<td>5.130</td>
<td>3.710</td>
</tr>
<tr>
<td>25% Percentile</td>
<td>14.05</td>
<td>13.16</td>
<td>14.56</td>
<td>4.385</td>
</tr>
<tr>
<td>Median</td>
<td>17.05</td>
<td>15.36</td>
<td>16.11</td>
<td>4.930</td>
</tr>
<tr>
<td>75% Percentile</td>
<td>18.20</td>
<td>17.47</td>
<td>16.87</td>
<td>5.670</td>
</tr>
<tr>
<td>Maximum</td>
<td>21.61</td>
<td>20.67</td>
<td>17.91</td>
<td>7.110</td>
</tr>
<tr>
<td>Mean</td>
<td>15.99</td>
<td>15.27</td>
<td>14.80</td>
<td>5.069</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>3.657</td>
<td>3.206</td>
<td>3.816</td>
<td>1.015</td>
</tr>
<tr>
<td>Std. Error</td>
<td>0.7980</td>
<td>1.134</td>
<td>1.272</td>
<td>0.3385</td>
</tr>
</tbody>
</table>
Figure 6: A graphical representation of the individual data from Table 3, showing the mean and the standard deviation across randomly chosen strains.

These data suggest that there is significant variation in THC potency within some strains but not others. There are also some cases of irregularity or outliers in each strain set. These data provide information on the lack of homogeneity across a strain. In some cases, there may be an error of categorization: a sample of Harlequin might have been mislabeled as Blue Dream. There may also be a significant genetic drift or disparity within the genotype of cannabis considered to be a single variety. Further, it is unknown whether there is similar variability in the terpenoid profile, which also contributes to the user experience.

**Heterogeneity across production methods**

The cultivation of cannabis has accelerated over the last 25 years, and is grown all over the world for a variety of uses and in a variety of ways. Globally, these operations can be grouped into three categories: historic/traditional production, cultivation in the developing world for the developing world, and production in the developed world—primarily outdoor but increasingly indoor operations (Decorte et al. 2011). The increased demand for cannabis since the 1960s has provided economic incentives for optimization of growing conditions for the highest yield and maximum potency. Given the range of approaches, it may be difficult to distinguish good from bad growing, but there is no doubt that plants are highly environmentally adaptable and that just like the market and the growers, there is a
lot of heterogeneity. Even when conditions are intended to be identical, there will still be variation across a crop.

Cropping methods and breeding strategies also affect the potency of cannabis (Burgdorf et al. 2011; Pijlman et al. 2005). Over the last four decades, the concentration of THC and other cannabinoids has increased, which baffled Mehmedic and coworkers as they found the “potencies inconceivable” and attributed their high measurements to “scientific and statistical shortcomings” (Mehmedic et al. 2010). There is little doubt that the potency increase is associated with both genetic selection and increasing sophistication of horticultural practices, including lighting, fertilization, addition of carbon dioxide, control of light intensity and photoperiod, temperature control, watering, balancing the pH of the soil, hydroponic growing, “supercropping”, plant spacing and trellising, and growing media (Chandra et al. 2008).

**UV lighting as a factor in THC content**

Ultraviolet radiation plays a role in enhancing THC levels in cannabis. Lydon et al. (1987) showed that THC content could be increased with UV-B irradiation (280-320 nm). However, indoor growing facilities currently favor high-pressure sodium lamps (emitting at around 546-620 nm) and metal halide lights (400-700nm). Seven varieties of cannabis were seeded and grown under conditions common to commercial practice to determine whether the level of electrical power is a useful estimate for final yield, and to determine whether the observation of increased potency could be attributed to lighting regimes (Potter & Duncombe 2012). Conditions were controlled with regard to day length, temperature, and CO₂ level. Zones of light with regard to electrical power consumption were varied and kept at a constant distance from the plant canopy as they grew for eight weeks. Flowers were then harvested, dried, and analyzed. Flower to leaf ratio significantly increased as a function of electrical power with an average yield of 470g/m². The authors did not report a significant difference in THC content based on this sodium lighting intensity however, and suggest that the increase in THC is based more on the breeding (genetics).

Table 4: The effect of light power density on Δ⁹-THC potency.

<table>
<thead>
<tr>
<th>Electrical Power Per Unit Area W/m²</th>
<th>Variety</th>
<th>Mean*</th>
</tr>
</thead>
<tbody>
<tr>
<td>270</td>
<td>Early Pearl</td>
<td>9.54</td>
</tr>
<tr>
<td></td>
<td>G1</td>
<td>10.49</td>
</tr>
<tr>
<td></td>
<td>Wappa</td>
<td>19.28</td>
</tr>
<tr>
<td></td>
<td>White Berry</td>
<td>11.04</td>
</tr>
<tr>
<td></td>
<td>Super Skunk</td>
<td>18.89</td>
</tr>
<tr>
<td></td>
<td>Hindu Kush</td>
<td>12.22</td>
</tr>
<tr>
<td></td>
<td>White Widow</td>
<td>17.78</td>
</tr>
<tr>
<td>400</td>
<td>Early Pearl</td>
<td>9.43</td>
</tr>
<tr>
<td></td>
<td>G1</td>
<td>11.07</td>
</tr>
<tr>
<td></td>
<td>Wappa</td>
<td>19.05</td>
</tr>
<tr>
<td></td>
<td>White Berry</td>
<td>10.45</td>
</tr>
<tr>
<td></td>
<td>Super Skunk</td>
<td>19.37</td>
</tr>
<tr>
<td></td>
<td>Hindu Kush</td>
<td>12.72</td>
</tr>
<tr>
<td></td>
<td>White Widow</td>
<td>17.53</td>
</tr>
<tr>
<td>600</td>
<td>Early Pearl</td>
<td>9.54</td>
</tr>
<tr>
<td></td>
<td>G1</td>
<td>11.36</td>
</tr>
<tr>
<td></td>
<td>Wappa</td>
<td>17.77</td>
</tr>
<tr>
<td></td>
<td>White Berry</td>
<td>11.02</td>
</tr>
<tr>
<td></td>
<td>Super Skunk</td>
<td>19.08</td>
</tr>
<tr>
<td></td>
<td>Hindu Kush</td>
<td>13.26</td>
</tr>
<tr>
<td></td>
<td>White Widow</td>
<td>17.43</td>
</tr>
</tbody>
</table>

* There was no observed increase in mean potency—linear regression, p > 0.05.

THC, Δ⁹-tetrahydrocannabinol.

Indoor growers often use a variety of lighting sources (including metal halide and LED) that provide greater spectrum of lighting, and measure lumens (not wattage) to predict vegetative growth. Whether and how specific wavelengths and intensities factor in THC
potency remains unknown. These are much needed experiments that could benefit the industry by maximizing product consistency and quality control.

**Degrees of plant/crop heterogeneity**

Indoor cultivation offers an advantage to the grower by allowing greater control over plant environment, and gives the ability to grow continuously without seasonal limitation. The setups vary widely with regard to sophistication. Typically, larger scale operations require higher levels of sophistication. It is assumed that more sophisticated operations are better able to regulate growth. However, variables such as nighttime temperature (if using outdoor ventilation), moisture and nutrient supply (if not automated), and equipment failures (fans, heaters, and coolers) can all contribute to outcome variability. In order to ascertain variability in yield and potency for criminal sentencing purposes in New Zealand, an initial study of crops of six plants in each of three “grows” were cultivated under controlled, indoor hydroponic conditions (Knight et al. 2010). Since environmental and nutritional factors were controlled, the study found that plant variety had a major influence on THC levels. A much wider study would be required to determine whether there is considerable variation in THC levels in a subspecies. Variability was determined by the authors to be due to flowers not all being at an equal stage of ripeness, and they recommend multiple analyses. A limitation of the study with regards to results from “grow 2” and “grow 3” were that they encountered serious problems, e.g., nutrient burn and spider mites, yet also found considerable variation both inter- and intra-plant. Individual data for the figure below was not provided, so the actual relative variability amongst this set of six clones is unknown (Figure 7).

Figure 7: THC results for six random samples from each plant in Grow 2, (clones) of the Knight study.
Overall, it can be assumed than even when growing the same variety under the same conditions there may be a substantial degree of cannabinoid variability. Based on the limited data available, it is best to label cannabis potency as a range - not a definite value, - for a given variety. Many laboratories currently report potency to two decimal points, allowing the consumer to misperceive precision for accuracy. This practice is misleading, and misrepresents the accuracy of testing protocols. Typically, that amount of specificity is warranted when results are based on multiple samples (n=3 in research). For single samples, decimal points should be dropped when reporting test results even if a lab has demonstrated a high degree of proficiency, as the inter and intra-plant variability warrant reporting potency in a range. Based on these data, a suggested range that may be reliable is around 2%. As the skill level of growers is refined, and with experimental data demonstrating a lower relative variation, this could be reduced to 1%. A state-sanctioned laboratory to conduct such experiments is needed.

Tensions in Testing Procedures

Many of the sampling policies described in this paper have significant implications on the price of testing. (To be clear, by price of testing we mean the costs levied on the producer, processor, and testing laboratory, as a result of specific policies regarding sampling methodology.) Since these regulations pertain only to the I-502 market, and not to the medical or black markets, minimizing the cost burden of testing and sampling-related policies is important to strengthening I-502’s ability to complete with these markets on price. Moreover, and for the same reason, even the most relaxed and least imposing testing and sampling policies discussed in this document will represent a cost increase over the current levels of testing expenditure as enjoyed even recently by the medical and gray markets. (However, these quality assurance regulations may also produce value, if consumers are willing to pay higher prices for cannabis with these assurances of potency and purity.)

The definition of a lot may have to evolve over time as the economy of scale evolves with the market. If it is projected that only large producers will be able to remain in the marketplace, then lot sizes will become much larger than what the existing framework may allow.

The suggested amount for a lot of plant material is not more than five pounds, based upon the approximate flower yield from an indoor grow facility using tables, or a 25 foot greenhouse row with mature plants spaced six feet apart (average yield estimated at 500 grams of flower per plant; Potter et al. 2012). The size of the greenhouse or area in which a particular variety is grown should determine what makes up the lot. A lot can be part of a larger unit that is a complete harvest. For instance, a harvest may include one lot or ten lots.

The decision on a maximum lot size entails a specific trade-off between cost and representativeness of the sample. As allowed lot sizes increase, producers and processors may separate products into a lesser number of individual lots, and testing laboratories may run fewer tests. Depending on required lot sizes, and based on the estimates of lot sizes in this document, a producer who produces a ton of plant material a year might have to pay as
much as $65,000 a year for the required testing (excluding the possibility of bulk discounts). This would represent approximately 4% of gross income. (See Table 5).

Another option to reduce the cost burden of these policies would be to allow growers to access semi-quantitative methods for potency results, and provide this along with pesticide testing data as part of the grower’s certificate of analysis. Then, producer-processors would absorb some of the costs for quantitative potency and microbiology testing of product that will be distributed for retail sale. For instance, HPTLC or infrared (IR) can be used to estimate potency and help growers conduct their own experiments with growing methodologies and harvest times. A semi-quantitative result can qualify for a certificate of analysis for the producer/processor. At the next stage of packaging and finishing the product, the quantitative analysis could occur (HPLC, GC).

Finally, we might expect some decrease in the cost of testing as the volume of demand for testing increases. For instance, a common blood test for total cholesterol has a retail price of five dollars in California, yet preparing a blood sample is more time-consuming and expensive than preparing a cannabis sample. One important factor that distinguished the cholesterol test from the cannabis test is the volume of sales activity to the vendor. As demand for testing increases, testing companies will be able to make more efficient use of capital and overhead, and thus costs for testing may sink across the board.

**Financial Feasibility for Raw Plant Material**

Twenty grams per kilogram from the producer equates to a net loss of an estimated 60 dollars in sales for the grower, or about 1% of the total lot price. With large-scale growing facilities, a 2% sample represents a cost of about $100, and at this time with the grower performing all of the required tests, another $200. $300 per kilo of plant material over a year, if producing a ton would cost the grower about $125,000 a year. If the lot size is increased to 5kg, costs would be reduced, but it will be necessary for laboratories to decrease the cost of running the test by improving high-throughput procedures. Estimates are provided in Table 5.

Table 5: Comparing the cost of testing cannabis flowers with respect to size of the grow facility.

<table>
<thead>
<tr>
<th>Lot (Kilos per year)</th>
<th># of 2 kilo batches</th>
<th>Sample cost per lot (@$3 per gram)</th>
<th>Test Cost per batch</th>
<th>Cost per harvest</th>
<th>Cost per Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.6</td>
<td>7.8</td>
<td>$468</td>
<td>$1560</td>
<td>$8,112</td>
<td>$64,896</td>
</tr>
<tr>
<td>9.36</td>
<td>4.68</td>
<td>$280</td>
<td>$1,216</td>
<td>$4,864</td>
<td>$38,937</td>
</tr>
<tr>
<td>4.68</td>
<td>2.34</td>
<td>$140</td>
<td>$608</td>
<td>$2,432</td>
<td>$19,468</td>
</tr>
<tr>
<td>2.34</td>
<td>1.17</td>
<td>$70</td>
<td>$304</td>
<td>$1,216</td>
<td>$9,734</td>
</tr>
</tbody>
</table>
The above table calculates cost for a producer with eight harvests a year (twice-annual production for each of four varieties, operating at different scales of production. Each test that must be carried out has two cost components: price of testing and value of the destroyed sample. The price of each individual test (including cannabinoids, pesticides, heavy metals, and microbiology) is assumed to be $50. Each test requires homogenizing 20 grams of product; in the testing process, seven grams are rendered unusable and the remainder may be returned to the producer in a homogenized state. The table assumes that the value of each gram of cannabis to the producer is $3 per gram, and that homogenized cannabis loses half of its value. (By these calculations, each test costs the producer a combined $40.50 in inventory.) Based on these numbers, the total cost of testing is 4% of the total potential gross receipts at $3 per gram.

Financial Feasibility for Extracts and Infused Products

It is more difficult to project costs of sampling extracts or cannabis-infused products. These are currently being produced in a variety of ways, from very expensive supercritical CO₂ extraction to simple tincturing with ethanol. Moreover, extractions are performed on widely different scales, from quart jar operations in home kitchens to larger lots in professional facilities. The amount of starting material and volume produced will vary greatly across these methods. Retail price also varies based on the cost of the materials involved in producing the product. A great deal more research should be done to determine representative sample sizes and cost projections for testing these products. More concentrated resins may need a smaller representative sample than a more dilute tincture. As discussed earlier, a representative sample from a lot may be 2% of the total or an AQL protocol can be developed that is based on lot size. Without knowing the exact costs of producing the various types of products, it is difficult to estimate the testing costs and feasibility.

A pound of raw material may yield about 50 g of a semi-solid extract using CO₂ extraction. For a tincture using glycerine or ethanol extract, the starting material will dictate the size of the lot. Standard statistical sampling was described in the sampling section.

Conclusion

There are many factors that can affect the cannabinoid profile and potency of cannabis. Controlling for the strain or genetic make up of a plant is often considered the most effective way to ensure a homogeneous crop. Though the WSLCB regulations require that a lot of cannabis be of the same strain, in this paper we have seen that other factors such as lighting quality and nutrients may play a major role in the potency of the plant. Informing the consumer about the strain of cannabis they are purchasing may not give them as much information about the psychoactive content of the product as could be hoped. Continuous testing of harvests is required to truly inform the customer.

Just like any other industry, standardized statistical sampling methods for the Washington cannabis industry are needed to ensure customer safety and to support a supply chain to produce products of unrivaled standards, purity, and quality. Cannabis is a highly variable
crop, and lot size must be small enough to recognize the unique makeup of a particular harvest. The extent of variability in cannabis is not infinite and at a certain point there are diminishing returns of reducing lot size. Required methods for gathering lots and retrieving samples must attempt to reduce variability and any opportunity for the results to be manipulated while at once keeping down the cost of testing.

The various methodologies and constraints of sampling methodologies have been explained herein. It is expected that some of these may change with the development of technology and the dissemination of knowledge across industry. The future trajectory of these developments has an element of unknowability, and there may come a time when they may merit a separate response from regulating agencies. In the meantime, additional research may be productive to the mission of ensuring the quality, consistency, and accurate labeling of cannabis products.
References:


BBC Research, “Global Market For Botanical And Plant-Derived Drugs Worth $32.9 Billion In 2013”, BCC Research LLC


