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Methods for Producing and Testing Extracts and Infusions

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I-502 Project #430-1d
August 24, 2013
Final

BACK OF THE ENVELOPE CALCULATIONS

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Introduction

This paper considers the issue of extracting and infusing cannabinoids from three perspectives: (1) best practices for performing the extraction and infusion process; (2) feasible methods for detecting inferiorities and/or dangerous residual solvents; and (3) miscellaneous issues.

Marijuana-infused products will constitute a significant portion of sales at I-502 licensed stores. Although these products contain the same psychoactive chemicals as does useable marijuana, they involve production processes and some public health concerns entirely distinct from those of useable marijuana. There exists a wide range of marijuana-infused products, of methods for extracting cannabinoid content from marijuana and for infusing that content into food and beverage, and of potential health hazards from their use.

Part one reviews the complexities involved in the extraction process. The section begins by identifying the various methods for extracting cannabinoids from marijuana and discussing each method's required levels of practitioner competency and capital, health and safety risks, and quality of output. The section also reviews the different chemical solvents used in the extraction process and their levels of harm to the consumer, in the case of consumption by the consumer of their chemical residues. (Of the many solvents used in the extraction process, some are quite toxic and others entirely benign.) The section closes by identifying the barriers faced by practitioners in obtaining high-quality and safe chemical solvents, due to the quasi-legal nature of Initiative 502 licensees and lack of standard scientific credentials.

Part two advances the discussion to the issue of infusing cannabinoid extracts into food or beverage. This topic includes the potential sources and consequences of error involved with infusion, such as incomplete decarboxylation, inadequate supply of solvents, and poor storage.

Methods of testing for potency and purity are also quite different for marijuana-infused products and extracts than for useable marijuana. Part three discusses the availability of potency and purity testing for these products, focusing on the ability of I-502 licensees to access these services in the short term.

Part One – Extraction Methods and Risks

I. Methods of Extraction

Chemical extraction of cannabinoids can be done in a variety of manners with varying selectivity for the target compounds. Historically four solvent-methods have been employed: (1) compressed liquid hydrocarbons such as butane; (2) supercritical fluid extraction; (3) uncompressed liquid solvents such as ethanol, methanol, acetone, 'naphtha'; and (4) very simple food-grade solvents such as oil, butter, and glycerin.

Many practitioners follow procedures that have been disseminated through Internet forums, with little regard for safety or effectiveness. In recent years, professional chemists have developed safer and more effective methods, such as supercritical fluid extraction, and some refining steps for a higher quality final product.

1. Compressed Liquid Hydrocarbons

Compressed liquid hydrocarbon extraction methods vary significantly in terms of health, safety, cost, and process. Further, liquid hydrocarbon systems come in both closed loop and open loop varieties.

1a. Open Loop Liquid Hydrocarbon

Open loop liquid hydrocarbon is a method commonly described on Internet forums. This process is also the cause of tragic fires and explosions in the homes of hobbyists and unskilled operators. The operator makes a canister out of a copper tube and packs it full of cannabis product. Then, using a can of either compressed butane (often a cigarette lighter refilling canister) (Ronson, 2011), propane, or propylene (Worthington), the compressed liquid is released into the semi-closed tube and sits for several minutes, before draining out the golden cannabinoid-infused liquid. Finally, the extract is placed on a hot plate set on low heat and the solvent is evaporated away, leaving behind cannabis resin. Cannabis resin is also known as cannabis oil or concentrate. It is the hydrophobic sticky, yellow/brown resin that is extracted from cannabis. It has the consistency of pine sap or cold molasses and is not water soluble.

There are high risks of explosion during the evaporation phase, especially in the hands of unskilled operators. When conducted in a household environment replete with spark-producing electric appliances, this process can generate large-scale fires; it only takes one spark to ignite the resulting flammable solvent vapor, creating a potentially lethal explosion and fire.

Low-quality (anything less than 95% in purity) butane or propane represents another area of public health concern; the less pure a source of butane, the greater likelihood it contains toxic contaminants in dangerous concentrations (FDA, 2013b). Many butane refill canisters contain 97.5%-99% pure butane with the major contaminants being propane, propylene, or a mixture of butane, iso-butane, and propane (FDA, 2013c). Of particular concern is propylene, which the FDA seems to not have approved for any use (it

is best known industrially as a precursor to isopropyl alcohol). Although propylene may be unsafe, it is highly available to the prospective hobbyist extractor. (Most hardware stores stock it with plumbing supplies, adjacent to propane torches and tanks.) Most users do not seek out a Material Safety Data Sheet[1] prior to use to see exactly what components are in the canister they are using, much less the purity or toxicity of the components.

This method has a very low entry-level price. The combined price of the copper tube, butane, and ancillary equipment runs around $20.

1b. Closed Loop Hydrocarbon with Reclamation Pump

With the development of the medicinal marijuana market, more professional, refined techniques of liquid hydrocarbon extraction have been developed. (Thanks in part to the efficiency and purity of butane extractions.) Enter "closed loop" processes, which are more expensive than open loop hydrocarbon extraction but provide for a more controlled extraction process and a safer end product. Depending on the efficiency of the model, the end product can still contain residual solvents, which should be removed by a vacuum oven or other method.

Some closed loop systems include a reclamation pump, which in combination with a heating system; manage to extract large amounts of cannabis extract with extremely low levels of residual solvents in the final product. One model like this routinely creates extracts with less than 200 ppm of the extraction solvents.

Closed loop systems have a fairly high entry-level price of approximately $20,000.

1c. Tamisium

Closed loop systems without a reclamation pump are called tamisia. Rather than using a reclamation pump, tamisia use externally applied heat and cold to passively move the solvent from one vessel to another while passing through the plant material. Compared to a closed loop, reclamation pump system, this produces resin with much higher levels of residual solvent.

This residual solvent must be removed by heat and/or vacuum. The residual solvent represents a safety risk for operators and a health risk for consumers. For operators, attempts to remove the hydrocarbon-soaked extract carry a significant risk of explosion. For consumers, the risk concerns the health impacts of any hydrocarbons that the extractors fail to remove. End users choosing to ingest rather than smoke or vaporize extracts are at special risk, since the hydrocarbons are not burned away before consumption.

Entry level pricing for a tamisium is around $4000.

[1] An MSDS is intended to provide workers and emergency personnel with procedures for handling or working with that substance in a safe manner, and includes information such as physical data (melting point, boiling point, flash point, etc.), toxicity, health effects, first aid, reactivity, storage, disposal, protective equipment, and spill-handling procedures.

2. Supercritical Fluid Extraction

Supercritical fluid extraction (SFE) is a very safe and effective method commonly used for decaffeinating coffee and creating other herbal extracts, and only recently applied to cannabis. In this case, the "supercritical fluid" refers to carbon dioxide (CO_2). According to this method, the operator packs a stainless steel extraction vessel with cannabis, seals it, and then fills the extraction vessel with CO_2 from a tank. At this point, the CO_2 is in its compressed, liquid form. The vessel is then pressurized to a supercritical state, in which CO_2 becomes neither gas nor liquid; it lacks any surface tension and mixes with other materials at extremely high levels of solubility. The cannabis is immersed in this condition for several hours, as the cannabinoids dissolve into the supercritical CO_2. When dissolution is finished, the CO_2 is slowly bled off and recompressed into the gas cylinder, while the extract is held in a collection vessel.

This method, while slower and requiring high power usage when compared with liquid hydrocarbons, has some advantages not seen in the other historically used methods, namely operator safety and non-hazardous solvent use. Devices for sale include closed loop systems starting at $60,000 and open systems that do not reclaim the CO_2 for $20,000.

3. Liquid Solvent

These methods use liquid hydrocarbons as solvents. Chosen solvents often include naphtha (popularly used to make Rick Simpson Oil), acetone, isopropanol, methanol, ethanol, denatured alcohol, and hexane. These solvents are often selected because of their ready availability, not safety or efficiency. Although many of these options are chosen by amateur extractors, ethanol is the best choice in regards to safety, low toxicity, and efficiency.

In this procedure, plant material is submerged in solvent and agitated, causing the cannabinoids to leave the plant material and go into solution. Once the cannabinoids have been sufficiently dissolved off the plant material, the plant material is filtered out of the solvent.

The next step is to remove the solvents from the cannabinoid extract. Some methods merely evaporate the solvent into the environment; this can be dangerous, particularly when performed with improper equipment and low skill. For instance, small-scale operators often use a hot plate or crock pot to vaporize solvent. (This generates a risk of fire for the same reason as discussed in the section on open loop hydrocarbon extraction; flammable gases are released into an environment with potential for ignition.) Variations on this method are illustrated in part by many videos on the Internet.

By some methods, such as distillation, these solvents are actually recovered for re-use in future extractions. Large-scale and professional operators usually use solvent recovery systems, which are both safer and more economical. With minimal cost and on a small scale, this can be accomplished with a tabletop unit intended for distillation of alcohol or herbal extracts, available on the Internet. Albeit at higher costs, a laboratory-grade vacuum distillation device is preferred. This is composed of a recirculating chiller, a hot water bath, a vacuum pump, and usually a motor to rotate the evaporation vessel.

An alternative method, as suggested by some internet forums, is a soxhlet extractor. This process continuously boils solvent then condenses it over the plant material until the material is submerged, then cycles the infused solvent back into the boiling vessel.

In the hands of a skilled chemist, any of these processes can produce safe and high quality extract in a highly safe manner. An unskilled hobbyist, on the other hand, might not manage to correctly follow the rather complicated procedures and processes. This would likely result in an end product of lower purity and a riskier production process.

The costs here are dominated by the equipment for a vacuum distillation process. The equipment required for the "rotovap" (rotary evaporator) method of vacuum distillation might be purchased for a minimum of $4,500 for used or up to $10,000 for new equipment. A vacuum oven alone might be available for $2000. On a small scale, a tabletop distillation unit would cost $200-$400 and is simple to operate. However, the cost of solvents and a jar to shake in would be less than $40.

4. Kitchen Grade Solvents: Butter, Oil, and Glycerin

This simple method does not require any skills or equipment outside the scope of the home cook. In this method, cannabis is submerged in melted butter or cooking oil in a large stockpot and warmed for any number of hours. Sometimes, water is added to help remove the chlorophyll and plant material from the slurry. Then the cannabis is filtered out using successive strainers and cheesecloth.

From an operator's standpoint, this is extremely safe, since there are no flammable gases involved, and the consumer does not risk exposure to hydrocarbons or toxins. However, the end product could have a higher bacterial load as a result of residual plant particulate, but frozen storage will reduce that risk. A drawback to these methods is primarily aesthetic. Color and flavor may impact final products negatively.

For more information on extraction terms, see Appendix 1.

II. Potential Health Harms of Ingesting Residual Chemicals

The following solvent discussion pertains to liquid solvents potentially used in the "liquid solvent" method of cannabinoid extraction. Most of these solvents may not be available to the general public, but are discussed in the US Pharmacopeial Convention chapter (USP, 2007) as chemicals commonly used in the pharmaceutical industry. U.S Pharmacopeia (USP) is a standard by which herbal supplements, vitamins, and over-the-counter (OTC) medications are tested for strength, purity, microbial load, consistency and function. USP makes recommendations and rules for the manufacture and storage of raw materials and extracts. USP divides popular solvents into three classes, based on their potential levels of harm to consumers (USP, 2007).

1. Class 1: Solvents to be Avoided at any Level (Table 10)

Solvents in class 1 should not be employed in the manufacture of cannabis or any herbal, extracts, excipients, and drug products because of their known or suspected carcinogenicity or their deleterious environmental effect (USP, 2007).

2. Class 2: Solvents to be Limited (Table 11)

Class 2 solvents are toxic and should appear only in limited concentrations. Permitted daily exposures (PDE) are given to the nearest 0.1 mg/day, and concentrations are given to the nearest 10 ppm. USP defines PDE as a pharmaceutically acceptable intake of residual solvents. The PDE calculation methods were established by methodologies and toxicity data from studies by the World Health Organization, U.S. EPA, and U.S. FDA. The equation to calculate the PDE for a particular solvent takes into consideration the no-observed-effect level (the highest dose at which there are no biologically significant increases in effects for exposed humans/animals), a weight adjustment, the extrapolation between species (should the study have been carried out on nonhumans to consider the comparative surface area to body weight ratios), the variability between individuals, and the length of the study and exposure among others. However, as no therapeutic benefit is derived from residual solvents, they should be removed to the greatest extent possible (FDA, 2009).

3. Class 3: Solvents with Negligible Toxic Potential (Table 12)

Solvents in class 3 may be regarded as less toxic and of lower risk to human health. None of these solvents are known as a human health hazard at levels normally accepted in cannabis extracts. Available data indicate that they are less toxic in acute or short-term studies and negative in genotoxicity studies. (Genotoxicity describes a damaging action on a cell's genetic material affecting its integrity.) According to USP rules, these residual solvents may be included in products without justification, so long as they appear at levels less than 50 mg per daily dose (corresponding to 5000 ppm, or 5mg/g, or 0.5%). However, many of them have not been studied for long-term toxicity or carcinogenicity; long-term study might find deleterious effects (FDA, 2009).

4. Levels of Consumption Required to Constitute Health Concerns

As long as residual solvent levels are kept below the concentration limits allowed by USP (Tables 10, 11, 12), consumption of cannabis extracts represent a low risk to consumer health. The concentration limits set by USP are calculated by the equation: $Concentration\ (ppm) = \frac{1000 * PDE(\frac{mg}{day})}{Dose\ (\frac{g}{day})}$. This equation assumes that a product weight of 10g is administered per day. Based on the concentration limits and PDEs, even a relatively dangerous extract, with high levels of toxic residual solvents, would have to be consumed in extraordinary quantities in order to exceed that chemical's PDE. This is true regardless of method of intake (although smoking and vaporizing represent a somewhat lower risk, since they may burn off any hydrocarbons prior to ingestion). Because cannabis extracts

are consumed in such low volumes, cannabis extracts could easily exceed permitted concentrations of class 2 and 3 solvents without causing the consumer to exceed PDEs or daily dose levels requiring specific justification.

The following calculation details the health risk of class 3 solvents. Assume one gram of extract, containing the maximum allowed concentration of class 3 solvent (5000 ppm, or 5mg/g), and a conservative THC concentration of 50%. In gross terms, this product contains 500mg of THC and 5mg of class 3 solvents. According to the 10mg serving size detailed in the I-502 rules, each serving contains 0.1mg of class 3 solvents. At this rate, a consumer could consume 500 servings of extract or marijuana-infused product before reaching levels for which the USP would require justification (USP, 2007).

Class 2 solvents generate more risk, but only slightly. For extracts containing methanol at the highest concentrations allowed by USP, a consumer would have to ingest 300 servings before breaching PDEs. Other commonly used class 2 solvents include hexane (29 servings per day allowed), acetonitrile (41 servings per day allowed), chloroform (6 servings allowed), and dichloromethane (60 servings allowed).

Environmental concerns are also at stake. In particular, chlorinated hydrocarbons (containing the class 1 solvents plus chloroform and dichloromethane) can be extremely hazardous to the environment, whether evaporated or poured down sinks or drains. This concern is negligible for extractors with solvent-recovery systems, which allow them to recover and reuse solvents repeatedly without negative consequences. However, extractors without recovery systems should enlist the services of legitimate solvent disposal services (Vanderbilt).

Table 10: Class 1 solvents in pharmaceutical products

Solvent	Concentration limit (ppm)	Concern
Benzene	2	Carcinogen
Carbon tetrachloride	4	Toxic and environmental hazard
1,2-Dichloroethane	5	Toxic
1,1-Dichloroethene	8	Toxic
1,1,1-Trichloroethane	1500	Environmental hazard

Table 11: Class 2 solvents in pharmaceutical products

Solvent	PDE (mg/day)	Concentration limit (ppm)
Acetonitrile	4.1	410
Chlorobenzene	3.6	360
Chloroform	0.6	60
Cyclohexane	38.8	3880
1,2-Dichloroethene	18.7	1870
Dichloromethane	6.0	600
1,2-Dimethoxyethane	1.0	100
N,N-Dimethylacetamide	10.9	1090
N,N-Dimethylformamide	8.8	880
1,4-Dioxane	3.8	380
2-Ethoxyethanol	1.6	160
Ethyleneglycol	6.2	620
Formamide	2.2	220
Hexane	2.9	290
Methanol	30.0	3000
2-Methoxyethanol	0.5	50
Methylbutyl ketone	0.5	50
Methylcyclohexane	11.8	1180
N-Methylpyrrolidone	48.4	4840
Nitromethane	0.5	50
Pyridine	2.0	200
Sulfolane	1.6	160
Tetralin	1.0	100
Toluene	8.9	890
1,1,2-Trichloroethene	0.8	80
Xylene*	21.7	2170

Table 12: Class 3 solvents that should be limited by quality-based requirements

Acetic acid	Heptane
Acetone	Isobutyl acetate
Anisole	Isopropyl acetate
1-Butanol	Methyl acetate
2-Butanol	3-Methyl-1-butanol
Butyl acetate	Methylethyl ketone
tert-Butylmethyl ether	Methylisobutyl ketone
Cumene	2-Methyl-1-propanol
Dimethyl sulfoxide	Pentane
Ethanol	1-Pentanol
Ethyl acetate	1-Propanol
Ethyl ether	2-Propanol
Ethyl formate	Propyl acetate
Formic acid	Tetrahydrofuran

III. Availability of Solvents and Extraction Gases

Unlike butter, cooking oil, and glycerin, extraction gases are not readily available in the grocery store. While many average quality solvents and gases are available at the local hardware store, the products there are generally intended for industrial purposes, not for making something to be smoked or eaten by humans. The higher quality gas, the more difficult it may be to obtain. The more purified gases are vended at specialty gas retailers who supply all kinds of compressed gases to various industries. For example, oxygen for hospitals, propane for home heating, nitrous oxide for dentists, carbon dioxide for soda production, acetylene and nitrogen for welding, and butane and propane for laboratory work or botanical extraction. Because these vendors usually have industrial clients, it can be difficult for an individual or a professional cannabis extractor to establish a working relationship with these suppliers.

1. Sourcing of Extraction Gases

Extraction gases are widely available, in both high- and low-quality forms. High purity liquid hydrocarbons are available from local compressed gas retailers. These retailers have low entry-level requirements, but may balk at vending to a marijuana related business. To start, tanks to be used for storage and reclamation must be purchased. A twenty-pound tank with a dip tube for liquid uptake can be purchased for $150 each. The tanks must be sent to Texas for refilling, so it is common practice to have an extra tank. N-butane, iso-butane and propane are available in CP (Chemically Pure) grade (99.0% pure) and Instrument Grade (99.5% pure). Many gases are also available in Technical Grade (95%

pure), but this grade of gas for n-butane, iso-butane, and propane is not stocked as frequently. Filling an already purchased twenty pound tank with CP grade liquid hydrocarbon costs $230-$260. Depending on the density of the specific gas purchased, a customer would be buying twenty to twenty-four pounds. Filling an already purchased twenty pound tank with Instrument Grade liquid hydrocarbon costs $216-$300 (Norco, 2009).

Hardware store sourced compressed gasses include LP or liquid propane; used either for barbeque grills or smaller tanks for plumbing repairs. The plumbing soldering torch is at least 85% propane, up to 15% propylene, and up to 2.5% butane. It also includes ethyl mercaptan, the odorant additive which may have negative aesthetic effects on a final cannabis extract product. Also available in the plumbing department is a bottle of compressed propylene. No documentation verifies that propylene is considered GRAS., However, its price and availability could make it a viable choice for an uneducated extractor. While widely available, these gases are poor choices for extraction due to their low purity. Other dangerous solvent sources include barbeque grill tank exchanges. These vendors offer low-grade propane, but at much too low a purity grade to be used for consumable products.

2. Sourcing of Liquid Solvents

In the unregulated gray and black markets, marijuana processors choose solvents based primarily on availability. Unfortunately, access to high-quality solvents is quite limited to cannabis extractors, whether or not they possess an I-502 license.

Scientific supply houses (such as VWR, Fisher Scientific, and Cole-Parmer) have these solvents available at accessible prices and in high quality. However, sales of these chemicals are extremely restricted, due to policies aimed at limiting supplies for methamphetamine labs and other types of illegal manufacture. Unfortunately for I-502 businesses, marijuana extraction businesses fall under this umbrella of illegal activity. These policies make it extremely difficult, if not impossible, for I-502 licensees to purchase high-grade solvents at scientific supply stores.

Local hardware and home improvement stores provide wider access to solvents, although at highly variable qualities. Solvents typically on stock at such stores include naphtha (aka Rick Simpson Oil, containing 95% hexane) and denatured alcohol (approximately 48% ethanol, 48% methanol, <4%methyl isobutyl ketone). Solvents marked as food-grade are generally of higher purity than their industrial counterparts, even if neither are intended for marijuana extraction. Food Grade ethanol is available in large volumes from distilleries. By obtaining a Class 2 permit from WSLCB (WSLCB, 2013b), a processor is capable of buying larger volumes (one to twenty-seven gallons from one supplier) (Alchemical Solutions, 2013). Even more accessible is vegetable glycerin, available online for $1-$3 per pound before shipping, and in one or five gallon sizes. Vegetable glycerin is an inefficient extraction solvent, but is a useful base for the final product used for dosing. Cannabis extract is quite soluble in propylene glycol. While this is helpful for some uses, the flavor can be a deterrent for use in large quantities.

Part Two - Dosing and Infusion Programs and Best Practices

Many consumers eat cannabis-infused foods or beverages. It is important to have an accurate and precise dose for an edible product. Once consumed, the amount of THC in the body cannot be adjusted. Cannabis extracts should be decarboxylated (THCA which is non-psychoactive converts into THC which is psychoactive by losing CO_2) before infusion. An extract with THCA still remaining can lead to a higher dosage of THC than desired, due to the ready decarboxylation of THCA over time.

I. Infusion Practices

Before an extract is used for infusion into a food or beverage, it should be decarboxylated. Decarboxylation is the process by which THCA (non-psychoactive form of THC naturally occurring in the plant) is converted into THC (psychoactive). While this conversion can happen unassisted over time, infused product makers will usually use a controlled heating process to activate the extract. Heating an extract at 150˚C for 10 minutes, or at 140˚C for 45 minutes, will usually successfully decarboxylate the extract. An extract should be analyzed by a cannabis testing laboratory both to assure full decarboxylation and to determine the potency of the extract. (A product may be considered fully decarboxylated if at least 95% of its cannabinoids have been converted into decarboxylated forms; it is unrealistic to expect 100% conversion.) This potency determination will allow the infused product maker to add the proper amount of extract to a batch of infused edibles to achieve a specific dosage.

An infused product manufacturer will produce an extract in a final form that is most easily miscible into a given edible product. Producers often choose the form of the extract based on compatibility with the intended matrix. For instance, cocoa butter is often used for infusing chocolate; ethanol-based extract is often used to make tincture or candies; olive (or other) oil or butter are used to make baked goods; finally, propylene glycol is often used to make vapor pens.

Some infused product makers infuse on such a large-scale that they simply weigh extracted resin into a batch of whatever product they are making. However, it can be difficult to handle and accurately weigh out concentrated resin, due to its thick and sticky consistency. Using a carrier such as those listed above (ethanol, cocoa butter, olive oil, butter, glycerin, and propylene glycol) helps mix the THC more homogeneously into a batch of edibles. Having a quantified, dosed carrier also dilutes the THC to a point where it can be much more precisely measured into a batch.

II. Potential Errors / Repercussions of Improper Infusions

Several errors can occur during the dosing of infused products. Steps to avoid these errors can be taken.

1. Calculation Errors

Navigating the conversion between metric and Imperial units and understanding the difference between milligrams, grams, and milliliters can introduce calculation errors into the dosing process. While mathematical errors can be inherent in any process, documenting the calculation steps on a production log and inserting some checking and confirmation steps into the production process can go a long way towards eliminating costly and potentially dangerous math errors.

2. Decarboxylation Errors

Not completely understanding the impact that temperature can have on the cannabinoids can lead to decarboxylation errors. A manufacturer may use a process that is so hot; the cannabinoids actually decompose, leaving an undosed or underdosed product. Some manufacturers do not decarboxylate at all prior to dosing a batch of dough to be baked, counting on the baking process to completely convert the THCA. This sort of procedure does not take into account the temperature differential between the oven cavity, the surface of the baked good, and the interior of the baked good. Often this practice will result in partially activated products or mistakenly deactivated products. These practices persist because potency testing of finished goods is only a newly mandated practice. (For more information on testing practices and facilities, see Appendix 4.)

In order to detect incompletely decarboxylated products, marijuana-infused products and/or extracts should be tested for Delta9 THC and for THCA (see Appendix 2 for more on cannabinoids). A goal of 95% decarboxylation of THCA to THC is attainable and desirable. This leaves the end user with a stable solution, with known psychoactivity. Otherwise, product potency is liable to increase as its cannabinoids decarboxylate while the product sits on shelves awaiting sale. (Incomplete decarboxylation is discussed in greater detail in "Testing for Psychoactive Agents" (Habib, 2013) and Part Three – Testing for Psychoactivity, Residual Solvents, and Contaminants.)

Incomplete decarboxylation should be managed in the regulatory guidelines. THCA is an unstable compound. It will tend toward a more stable form by losing a CO_2 molecule (decarboxylating) and becoming THC. It will do this over time, even at room temperature, in a candy or cookie. If an extract is only 50% decarboxylated, then the final product could have 10mg THC, and 10mg THCA. Over an unknown amount of time this may become a 20mg THC edible. Illustrating this incongruity in another way, if an edible is advertised as a 10mg edible but has 5mg THCA and 5mg THC, the consumer will not get the level of psychoactivity expected from a 10mg product. This will alter their perception of what a 10mg product feels like, potentially compelling them to buy a stronger than necessary infused product next time. Even more risky, a manufacturer could create a frozen dough product with 10mg THC and 40mg THCA and provide instructions to the end user that the product will really 'pop' if baked at 300°F for 15 minutes. Baking under these conditions could easily increase the THC level to nearly 45mg, as THCA converts into THC. Such a product might work around the 10mg-THC limit per serving for marijuana-infused products (WSLCB, 2013a).

3. Lack of Batch Accuracy

An infused product producer needs to precisely measure ingredients and hone their processes in order to get the exact number of servings at the correct size and dosage as expected. Currently, most untested edibles are merely cobbled together with no regard for accuracy or precision. Ensuring consistent and accurately dosed products reduces risks of both under- and overdosing. Although the costs of overdosing are obvious – manifesting in emergency room visits, higher risk for accidents, and perhaps increased chance for habit-formation – there are also risks associated with underdosing. Consumers might respond to underdosed products by raising their desired nominal dose; moreover, underdosing threatens the integrity of the potency labeling system and the value of the commercial marijuana market as a whole.

It has not been standard procedure in the black and gray markets for infused product makers to submit their products for potency testing, although it is done on occasion. In most circumstances, the cannabinoid content advertised on infused product labels is calculated via a more indirect method, if at all. Infused product makers will begin with a cannabinoid extract of known potency – either extracted themselves or purchased from an independent extractor – and carefully proportion this dose into the infused product.

When done competently, this process can produce infused products with accurate and consistent doses. Most infused product manufacturers emphasize they measure to the 0.01g when weighing out resin. Moreover, many reconstitute resin extract into an ethanol or oil base in order to provide more accurate measurement and better product assimilation. However, if the cannabinoid extracts used as ingredients are improperly tested (or of inflated potency, as often occurs in the current unregulated testing industry), the potency of the infused product will be symmetrically under- or over-dosed.

Experienced large-scale quality infused edible producers maintain a 10-12% precision goal for their batches. However, even competent small-scale producers can experience variability of 15%-20%. An attainable standard for even small-scale producers in the I-502 market may be ±20%, which would be in line with standards for other analogous industries such as food production and nutritional supplement production.

III. Other Concerns: Storage, Testing Protocols

Extracts of any matrix should be routinely stored under refrigeration for increased stability. Some infusions can be stored in the freezer, such as ethanol solutions or butter infusions. A processor could choose to extract the cannabis to produce a resin, then store that resin under refrigeration or freezing until infusing it into ethanol, oil, glycerin, or the carrier of choice, when needed for production.

Using this scenario, certain laboratory tests would be pertinent only at specific points in the process. For example, the resin should most certainly be tested for solvent residue if a liquid hydrocarbon or solvent was used to prepare it. But, if the resin is destined to be infused into a carrier, it makes little sense to mandate potency testing until the extract is infused into its final dosing form. In this scenario, there could be a 'mother' lot

of resin, which is infused into several smaller lots of infused extract solutions; ethanol, olive oil, propylene glycol, etc.

Part Three – Testing Availability and Cost

Extracted and infused products, like usable marijuana, ought to be tested for both psychoactive potency and harmful contaminants. However, these processes are different than that for usable marijuana in important regards. In terms of potency, the feasible range of concentrations is much higher, since concentrations are not limited by the cannabis flower's natural capacities for chemical production. Instead, use of different extraction and/or infusion processes can create infusions that are very low potency or concentrates that are quite the opposite. Secondarily, some extracts and infusions are sold with the expectation that the product has been fully decarboxylated, such that all THC-A and CBD-A has been converted into THC and CBD; other extracts and infusions may be deliberately sold and marketed in carboxylated forms. In terms of toxic content, extracts and infusions suffer an additional source of contamination: residual solvents. The danger here varies based on both concentrations and the particular chemical used as solvent (as previously discussed in Part Two). A third concern, as with all manufactured food items, is microbial growth. And finally, the fourth concern is the transfer of any chemical contaminants (pesticide, growth modulators, etc.) from the raw plant material to the extract.

Multiple tests can be done on cannabis products. Labs use different types of chromatography to test for potency and contaminants. The cost and sample size required for testing may deter some distributors from testing each batch of cannabis for potency and contamination.

For more information on testing facilities, see Appendix 4.

I. Testing for Psychoactives

Testing extracts for psychoactivity is a relatively low labor test, and according to a survey of testing labs, should cost around $50 and requires a 0.5g sample size. However, testing a finished edible product for potency can raise a host of complications. Extracting cannabinoids from a cookie or beverage involves separating the cannabinoids from sugar, flour, chocolate, and fat. A lab should do extensive method development with each kind of edible (baked goods, hard candies, beverages, etc.) to ensure that their extraction method is efficiently and completely removing all the cannabinoids from the edible. Additionally, once complete extraction is confirmed the sample must be 'cleaned' of other contaminants that may interfere with chromatographic analysis. This extensive method development and additional sample treatment expenses may make finished good analysis more expensive.

High-performance liquid chromatography (HPLC) is the preferred instrumentation recommended for edibles or extracts THC analysis. It allows for the separation, identification, and quantification of each component in a sample. It is important to differentiate between THCA and THC so that consumers can see the actual psychoactivity expected from the product. Using HPLC, the cannabinoids are not chemically modified, and therefore data on both the acidic and neutral forms can be collected.

Finished goods should be tested regularly, with a precision goal of +/-10%. Good manufacturing practices will assure consistent accuracy and precision. There could be a regular schedule of batch testing to confirm that finished goods are dosed consistently and correctly. There should be an acceptable range of dose accuracy, outside of which products are rejected.

II. Testing for Solvent and Chemical Residue

Tests for solvent residue for marijuana extracts are still in their infancy. Nationwide, fewer than five medical marijuana laboratories offer the service, and most of those offerings are still undergoing development. Where these tests are available, they often charge $75 per sample and require a 0.5g sample of extract (Haneman, 2013). For processors known to be using non-toxic solvents, such as CO_2 or Food Grade ethanol, residual solvent tests would be unnecessary.

If useable marijuana and trim material contains any chemical residues from pesticides, miticides, mycotoxins, or growth modulators, those chemicals can sometimes be extracted and concentrated into a cannabis extract. Any cannabis used for extraction should be tested prior to extraction for these chemical residues.

III. Testing for Microbial and Fungal Content

Finished edible goods should be tested for microbial load. Processors will need to learn and enforce food handling and packaging rules in order to successfully keep healthy products on the shelves and meet reasonable 'best by' dates. Some products have a higher risk of microbial growth than others. For example, a muffin will more likely mold than a hard candy. In addition to poor handling and packaging practices, the addition of dairy and higher moisture content increase the risk of microbial proliferation.

An extract made using solvent, liquid hydrocarbon, or CO_2 should be rendered sterile by the extraction process, and need not be subjected to particular tests for microbial and/or fungal content (Appendino, 2008).

Extractions performed with butter or oil extracts are not equally sterile. Bacteria readily grow on any plant material that remains floating in cooking oil, and butter itself can go rancid or moldy. A butter or oil extract that tests clean upon production still may host bacteria or grow mold over time, depending on how it is stored. The exception to this fecundity problem would be if an extract was made using a liquid hydrocarbon or CO_2 solvent and subsequently dissolved into oil, rather than extracted using oil. This product would carry lower risks for bacterial or fungal content.

Mold/bacteria testing as described in the Cannabis Monograph may be unusually costly if a 25g sample size is required for extracts (as indicated in the monograph) as well as finished goods. Instead, an extract could be tested as a representative sample of finished serving size.

Finished goods should be routinely tested for bacterial and fungal load regardless of what kind of extract they were dosed with. Products that retain a high amount of moisture are more conducive to microbial growth than drier goods.

Appendix 1: Extraction

1. **Butane** (open tube): This method is the use of a copper, or other material, tube with semi-closed ends packed with cannabis, into which butane is injected into one end and collected out the other. Butane is then usually evaporated in the open air (FDA, 2013c).
2. **Butter/oil infusion** (crock pot, stove top): This is a method of extracting cannabinoids from cannabis by submerging, heating, and mixing plant material with melted butter or warm oil. The plant material is removed using cheesecloth or muslin. There is often residual plant material in the extract.
3. **Closed loop system**: This is an extraction system that contains and collects the extraction solvent without exposing it to the ambient environment.
4. **Liquid hydrocarbon** (closed loop): This is an enclosed system, usually stainless steel including at least three basic parts: a tank for LH storage, an extraction vessel to hold plant material, and a collection vessel to hold extract. A valved tube connects the collection vessel and the LH storage tank. A system that uses external heat and cold sources to move the solvent is called a tamisium. A system that adds the use of a pump is simply a LH Extractor.
5. **Liquid hydrocarbon**: Some examples are butane, iso-butane, and propane. A liquid hydrocarbon is any solvent made of carbon and hydrogen atoms that is in a gaseous state at room temperature and pressure, but can be stored as a liquid in a pressurized tank.
6. **Liquid solvent** (shaken, soxhlet extraction, sonication): This is the use of a liquid form solvent in a jar or other vessel. One pours solvent over plant material and extracts cannabinoids by shaking or sonicating. The cannabis solvent is collected by filtering out the plant material. The solvent can be reduced or completely evaporated either in the open air by hotplate or can be recollected by using vacuum distillation.
7. **Stovetop evaporation**: This is the use of electric or gas stove to evaporate the solvent, thereby concentrating the cannabis extract. This method is very hazardous due to the creation of flammable or explosive vapors around household appliances.
8. **Supercritical fluid extraction** (carbon dioxide): This method uses liquefied carbon dioxide under high pressure to extract cannabinoids from plant material. Regular beverage grade CO_2 can be utilized, but high purity instrument grade CO_2 is better for the equipment. CO_2 can be reclaimed or harmlessly dispersed after extraction. This method has very expensive entry costs, but is very cheap to use. This process is often used to extract caffeine from coffee or many other active compounds from herbs or plants.
9. **Tamisium extractor**: This is a closed loop system in which the hydrocarbon solution is moved passively by heating to expand and cooling to condense into another vessel (Tamisium Extractors, 2013).

10. **Vacuum distillation**: This is use of a warm water bath, a vacuum pump and a chilled condenser used to evaporate and collect solvent while leaving behind cannabis extract. One version with a rotating flask is commonly known as a 'rotovap'. These are commonly used in laboratories for concentrating samples.
11. **Winterization**: This is the freezing of a solvent cannabis extract, causing plant waxes and other unwanted plant materials to fall out of solution. After winterization, the waxes are filtered out of the solution.

Appendix 2: Cannabinoids

1. **Cannabidiol** (CBD): This binds to the CB2 receptor. CBD is not psychoactive. It has been proven to have several therapeutic qualities including inhibiting some of the more unpleasant side effects of high doses of Delta 9 THC. CBD exhibits anti-inflammatory, anti-seizure, anti-cancer, and neuroprotective qualities.
2. **Cannabidiolic acid** (CBDA): This is the acid form of CBD. This acid form, like THCA, is the most prevalent form of CBD in fresh plant material. 90-99% of the CBD in fresh plants is found in this acid form. CBDA converts to CBD by losing a CO_2 molecule, most likely by heating/smoking the plant material.
3. **Cannabinol** (CBN): This is a degradation product of Delta 9 THC. It is not considered psychoactive or particularly therapeutic, although it has been shown to mildly stimulate the appetite.
4. **Delta-8-tetrahydrocannabinol**: This is an analogue of tetrahydrocannabinol (THC) with antiemetic, anxiolytic, appetite-stimulating, analgesic, and neuroprotective properties. Delta-8-tetrahydrocannabinol (delta-8-THC) binds to the cannabinoid G-protein coupled receptor CB1, located in the central nervous system. This agent exhibits a lower psychotropic potency than delta-9-tetrahydrocannabinol (delta-9-THC) (National Cancer Institute).
5. **Delta-9-tetrahydrocannabinol** (THC): This is the psychoactive component of cannabis. It is in very low concentration in fresh plant material, but is formed by the decarboxylation of THCA, most often by heating/smoking the plant material. It binds to the CB1 and CB2 receptors in the body. For more information of receptors and cannabis interaction with the human body see Appendix 3.
6. **Terpene**: This is a class of chemical found widely in nature. Most often, terpenes are the odiferous component of herbs and shrubs. They are extracted as essential oils. Cannabis has many terpenes that are common in other common herbs. Linalool is in lavender. Pinene is in evergreen trees. Limonene is in citrus rind. Betacaryophyllene is in black pepper. All of these are present in many strains of cannabis as well. While not psychoactive, they are presumed to be therapeutic in nature.
7. **Tetrahydrocannabinolic acid-A**(THCA): This is the A isomer of THC Acid. THCA is the most prevalent form of THC in the cannabis plant. It is not psychoactive. 90-99% of the THC in a fresh or recently harvested cannabis plant is in this non-psychoactive form. THCA converts to THC by losing a CO_2 molecule. This process is called 'decarboxylation' and is promoted by heat, UV, and oxidation.

Appendix 3: Human Interaction with Cannabis

1. **CB1 receptor**: This is a naturally occurring cannabinoid receptor located primarily in the central nervous system, but also in some peripheral tissues.
2. **CB2 receptor**: This is a naturally occurring cannabinoid receptor located exclusively in peripheral tissues, immune cells, and organs. It is not associated with the central nervous system
3. **GRAS**: This stands for generally recognized as safe. It is a category of food additives that have not gone under extensive scrutiny and review because they have been adequately shown to be safe under the conditions of their intended use (FDA, 2013a).
4. **Marijuana concentrate**: This is a physically concentrated form of cannabis. The active cannabinoids are all in the plant's trichomes: brittle, sticky resinous bulbs that form on the leaves and flowers of the plant. A concentrate is made by physically removing and collecting those trichomes. This is done using a silkscreen material and cold water (bubble hash) or by using very fine sieves (kief).
5. **Marijuana extract** (resin): This is a chemically extracted concentrate, made by using non-polar solvents, liquid hydrocarbons, or carbon dioxide. It is generally a gold to brown sticky resinous substance. It is used to make extract for dosing marijuana infused edibles or also for directly smoking.
6. **Marijuana infused beverage**: This is often a soda or "energy shot".
7. **Marijuana infused edible**: This is a food item in which cannabinoids have been added either by adding cannabis butter, oil, alcohol extract, or just straight extract.
8. **Marijuana infused liquid**: This is a tincture, sublingual spray, cooking oil, ethanol solution, or a glycol solution.

Appendix 4: Research Regarding Cannabis Testing Laboratories

	Do you test extracts for residual solvent? Which solvents do you typically test for?	Do you consult with your customers about calculating dosages?	Do you consult with your customers about decarboxylation on extracts to be used for edibles?	Do you test infused products, baked goods, candies, and/or beverages for potency? HPLC or GC? If GC, what kind of detector is used for solvent residue analysis? Which is more popular, testing of extracts or of infused goods? What is the ratio between the two in your lab?	Have you tested infused products for microbial growth? If yes, do you plate or use PCR?	What do you charge per sample? Do you charge more per sample for finished infused products than for extracts or plant material?
Analytical 360 (Analytical 360, 2011), (Randy Oliver, personal communication, August 7, 2013)		Yes we often provide them with the equations to calculate the final dilution and or other mass balance calculations.	Yes we often provide information regarding how to treat the sample to produce an activated product.	Use HPLC (more precise/efficient that GC). We test twice as many concentrates as edibles. 2:1 in the last 90 days.	Plate assay is used for microbial screening.	Potency or Microbial: $60/sample Potency and Microbial: $85/sample Not currently but due to the additional extraction time it would make sense to charge more.
GOAT labs (G.O.A.T. Labs, 2013)				Use both HPLC and GC. GC: SRI 8610GC with FID, DELCD, and NPD detectors HPLC: Agilent/HP 1050 HPLC qPCR: GeneAmp 9600 PCR	Use qPCR and micro-plate reader	
NW Botanical Analysis (NWBA, 2012), (Alex Prindle, personal communication, August 7, 2013)	Tests for butane (most frequent solvent for concentrates) to ppm	Yes/no. we provide info that allows them to dose the product appropriately. IE. we tell them what similar products are typically dosed at and we report results in mg/g, or mg/ml as well as in percentage of weight.	Yes, This is a basic chart that shows decarboxylation of a hexane extraction which is similar to other extraction methods, and explain the benefits of low temp, long time decarboxylation (terpene retention, full extraction, etc.).	Potency analysis using GC. Extracts. 5:1 Extracts:Infused edibles. Although, this isn't really representative as we don't do acid values yet so many of those clients use other testing facilities for those products.		Potency, pesticide, mold, butane and terpene analysis: $50 each. No, but we should. Not sure how things will react with HPLC Extractions as we just received our HPLC last week and are still familiarizing ourselves with it.

	Residual Solvents	Customer Dosage Consultation	Customer Decarboxylation Consultation	Infusions Potency Testing? Method? Ratio of Infusion to Extracts?	Infusions Microbial Testing?	Price per sample? Infusions v. Extracts v. Plant?
SC Labs (SC Labs, 2013)	Residual Solvent Testing: GC/FID, Head space analysis, MS Tests for: acetone, butane, propane, pentane, hexane, heptane, ethanol, isopropanol.			Potency testing using HPLC in flowers, concentrates and edibles GC causes acidic cannabinoids to break down into their neutral forms LC/MS used for pesticide testing Terpene Analysis uses GC/FID	qPCR used for pathogen testing Microbiological Screening: Real Time PCR (much faster than plating) LC/MS used for pesticide testing Terpene Analysis uses GC/FID	
Steep Hill (Steep Hill, 2013)				Analyze all cannabis products – flowers, concentrates, kief, hash, oils, tinctures and edibles Uses GCMS to test for potency HPLC and ELISA used to test for pesticides Equipment: Agilent 7890 GC/Flame Ionization Detector with 7863 Autosampler Agilent 5975 MS w/ EI Agilent 1100 series HPLC Spectramax 250 microplate reader Binder BD-240 Incubator	Use plates to count mold.	Sample size: 2 grams for potency screening, 2 grams for safety screening

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