Microbiological Safety Testing of Cannabis

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Introduction

Cannabis is increasingly becoming legal at the state level in the U.S., for either medical or recreational use. Each of these states has had to wrestle with the question of how to ensure the safety of a new product that is not covered under any existing safety guidelines. Safety testing in other agricultural industries is regulated by the FDA, the USDA, or by other federal agencies, all of which have been unable to assist the states in this case. The few states that have produced safety testing guidelines for Cannabis were forced to develop them from scratch, without the regulatory and scientific support that federal agencies typically provide.

In the absence of this federal guidance, regulators in each state have turned to different sources for information, and each state has produced a unique set of rules and regulations (if they have produced one at all). Many of these are in outright conflict with each other, and they are largely not grounded on scientific research. This whitepaper is focused entirely on the question of microbiological safety, and has been written in order to promote the adoption of regulatory guidelines for the Cannabis industry that are rational, consistent, and safe. We have gathered what data there are on this issue and related ones, and assembled a broad collection of experts on the general subjects of plant microbiology, medical microbiology, and safety-testing of agricultural and food products.

One reason for the difficulty that the states have had with this issue is the unusual delivery route of Cannabis. If Cannabis were not typically consumed by smoking, it would fall more clearly under existing guidelines covering pharmaceuticals or agricultural products. The only potential source of safety regulations pertaining to plant material consumed by inhalation would be the tobacco industry. However, that industry does not publish such information, and has only very recently been subject to any federal oversight at all. Regardless, the appropriate guidelines for this industry will need to take the delivery route into account very clearly. Inhalation presents a different set of health threats than does (for instance) oral ingestion.

The experiences of other industries supply a vast collection of both data and regulatory approaches from which to draw upon. Nonetheless, this industry is unique, and it will be impossible to develop the right regulatory approach without careful customization of the approaches used in other settings. The potential problems associated with a massive industry, arising practically overnight,

argue for stronger controls than are in place elsewhere. On the other hand, safety-testing guidelines that are too strict, economically unfeasible, or based on poor science, will be difficult to implement and will have a damaging effect on the industry as a whole.

Our approach in this white paper is try to balance these needs by giving recommendations that will exceed the public health protections in other industries, but not by more than is reasonable or necessary. More specifically, our approach has been to attempt to determine which microorganisms are likely to be present on Cannabis; which of them could potentially replicate to significant levels at any point in the production or use process; and which of these might actually pose a health hazard. We will recommend against testing for any organisms that do not meet these criteria.

Existing guidelines

Both Colorado and Washington require Cannabis to be tested for microbiological contamination. However, they have instituted very divergent rules for how to implement such testing. Washington State produced a set of guidelines based on recommendations in the American Herbal Pharmacopoeia's Cannabis Monograph[1]. These, in turn, were drawn largely from guidelines specified by the American Herbal Products Association (AHPA). Colorado included a list of microbiology testing requirements in the actual legislation establishing the legal Cannabis industry in that state. These included a list of microorganisms that testing would be required for, and this list was clearly drawn from the small existing literature on Cannabis microbiology. The initial Cannabis microbiology testing requirements for both of these states were as follows.

Washington

Total Viable Aerobic Bacteria Count	< 100,000 CFU
Total Yeast and Mold Count	< 10,000 CFU
Bile-tolerant Gram-Negative Bacteria	< 1000 CFU
total coliforms count	< 1000 CFU
E. Coli (pathogenic strains)	not detected in one gram
Salmonella spp.	not detected in one gram

Colorado

E. Coli	none detected
Salmonella	none detected
Gram-Negative bacteria	< 10,000 CFU
Aspergillus	none detected
Penicillium	none detected
Mucor	none detected
Thermophilic Actinomycetes	none detected

The AHPA guidelines, from which the Washington State requirements are drawn, specify a list of tests that are typical microbiology guidelines in some food products industries. They predate much of our modern scientific knowledge, and are based primarily around the use of testing techniques that existed at the time rather than actual data about relevant pathogens. The limits specified in this case were set based on surveys of the AHPA membership, and are thought to reflect actual knowledge about what levels are common for dried herbal products in general (Magad Sharaf, AHPA Standards Committee, personal communication). These limits therefore represent averages across many hundreds of different types of plant products. The number of total viable microorganisms can commonly vary by a factor of many thousands between plant types and across seasons and growing conditions.

Most importantly, as with all food-safety guidelines for agricultural products in this country, these were generally not intended as a testing protocol for each lot or batch of a product. Most existing approaches to food safety do not mandate testing of end products. Instead, except in cases of immediate public-health threats, they require testing and certification of production facilities and processes, even if this is accomplished in part by sampling of final products. This approach leads to a different set of recommendations for testing. It favors broad quality-indicator tests, and can reasonably include tests for organisms that have no special likelihood of being present. The AHPA guidelines are industry-specific self-regulatory guidelines, and as with similar lists in different industries, they were originally developed to represent average target levels. They were not intended as pass/fail criteria to be applied to each lot of product, and no regulatory body in this country requires that they be applied in such a way.

There are various reasons for requiring "indicator tests" that don't directly test for pathogens, but instead serve as "quality tests", or indications that follow-up pathogen testing should be performed[2-4].

Chief among these are their utility in monitoring production processes themselves. They are less useful, and much less utilized, in the case of end-product testing. The Washington State microbiology guidelines include four separate indicator tests. One of these (total coliforms) is no longer considered the best test in its category[5,6]; another (bile-tolerant gram-negative bacteria) is so outdated that most of the authors of this white paper were unfamiliar with its use in safety testing. In fact, in some documents it is listed as being functionally identical to indicator tests for "total coliforms". Total coliforms is another indicator test also present on the Washington state list. Indicator tests will be discussed in more detail below, but in the case that such tests are required, we recommend that they be kept to a minimum, and used only in cases where they will provide actionable information.

The initial Colorado regulations are quite different than the Washington state ones. The list of mold species, in particular, appears to originate with a series of papers on Cannabis microbiology published by one of the co-authors of the present white paper (JW McPartland)[7-10]. They are all molds that have been isolated at one time or another from Cannabis plants. However, spores of these species are ubiquitous, and they were mentioned in these publications in the context of experimental studies that assessed or replicated poor storage conditions. Studies of contaminants of marijuana in the 1970s and 1980s primarily investigated cannabis smuggled from Latin America. Their relevance to current, domestically-produced cannabis is very limited. The product was sweat-cured, then compressed into bricks for smuggling, under conditions not controlled for temperature or humidity. These conditions gave rise to "storage molds" that are easily discernable and frankly unacceptable by today's consumers. Some organisms reported in these studies, such as *Mucor sp.*, thermophilic *actinomycetes*, and *Dienerella arga*, indicated a highly deteriorated condition.

In another study that identified several of these mold species on Cannabis[11], the authors analyzed street samples submitted by cannabis smokers, and isolated *Aspergillus, Mucor*, and *Penicillium* species. The Cannabis in this study, as well, was likely smuggled into the country under inappropriate storage conditions. The study used nonselective culture media, which actually selects for the growth of fast-growing and ubiquitous fungi such as the *Mucor* and *Penicillium* species that were found. No quantification of these molds was provided, and allergy testing of each of the Cannabis smokers with *Mucor* and *Penicillium* antigens showed no greater sensitization

amongst smokers than control subjects.

At least one of these molds (*Aspergillus*) is a genus that does indeed contain species that are a health threat likely to be present on Cannabis. *Aspergillus* is ubiquitous in soil and on many plants, which means that the initial requirement in Colorado that there be "none detected" on Cannabis is not feasible. On the other hand, there are hundreds of *Aspergillus* species, and very few of these cause human disease, so a general test for *Aspergillus* is inappropriate. Another problem with requiring tests for these molds is that there is no adequate existing test that is specific to them. There is no selective media or commercial plate available that allows only these species to grow and be quantitated. Molecular methods have been developed for some of these species, but have not been generally commercialized. Non-commercial plate-culture methods do exist, but these require a trained mycologist to identify the mold species by eye in the presence of many different types of mold. Colorado has since modified its list of required microbiological tests, but it remains to be seen how they will approach many of these issues.

Microbiological growth conditions

In general, microorganisms can cause disease in two distinct ways. The first is through active infection: high-level replication in the host can lead to structural damage, toxicity, and dangerous hyper-activation of the immune system. The second is due to the fact that certain species of bacteria and fungi can produce toxins which can be ingested and cause disease even in the absence of viable bacterial or fungal cells. Toxin production itself requires robust replication; so although this disease mechanism does not require that microbial cells be alive or healthy at the point of ingestion, it does require that they were able to thrive on the food matrix at some prior point. It also requires that there be no processing step that removes or inactivates the toxin.

Likewise, active infection in the host typically requires high-level replication on the vector itself. This is because most infections are cannot be initiated without a large starting inoculum. Certain organisms are exceptions to this rule, and can initiate infections with extremely small doses – sometimes as little as a single cell or spore. Even these types of organisms (just as with toxins themselves) must of course also be able to survive any processing step that would kill them.

In short, many infections can be prevented by avoiding high-level replication that would lead to large infectious doses of viable cells, or to large doses of toxins. Many infections can be prevented by steps that kill or inactivate microbial cells or microbial toxins. If robust replication is blocked, and "kill steps" are implemented, then the only possible type of infection is by organisms that can survive existing kill step conditions and initiate infection with minute doses.

Both bacteria and fungi (i.e., mold) need permissive conditions in order to replicate and present a health hazard. They need a surface or matrix that they have evolved to colonize effectively. They need adequate nutrients, adequate available water, and specific temperature ranges. Because temperature and water requirements are the most critical for microbial growth, the most common steps that result in the killing of microorganisms on food products are heating and drying. Many food products are cooked in some way, and because high temperatures kill bacteria and fungi these foods are generally not a safety threat unless they are mishandled after the cooking process.

Bacteria and fungi also have very specific water requirements. Many plant-based foods have high moisture content and are able to support robust microbial replication. These pose a danger if they are not cooked properly. Modern microbiology safety standards take this into account. However, the potential for microbial growth is a function of "water activity" (A_W), and not of moisture content itself.[12,13]. Water activity is a measure of the available water that can be utilized for microbiological growth. It increases with moisture content, but it does so non-linearly, and in a manner that is unique to each material or matrix. High-moisture foods with high salt or sugar content can have quite low water activity, because the solute concentrations cause a majority of the water to be functionally unavailable. Water activity ranges from 0 to 1, and below A_w 0.6 no growth can occur. Most pathogens cannot grow below A_w 0.9; however some fungi can grow slowly at water activities as low as A_w 0.61[14].

The potential for plant-born infection depends on the temperature, water activity, and transmission-route parameters that characterize the particular product. For instance, smoked plant material is heated to high temperatures that will kill normal cells, but it can still deliver heat-resistant spores to the lungs. Edible food products are usually heated, but if they are not heated they can deliver bacteria to the stomach where certain species can replicate. The section below follows the Cannabis plant through the stages of growth, processing, and use,

in order to clarify what microbiological threats are possible at each stage.

Cannabis production and use

Cannabis can be grown in several different environments, processed in many different ways, and utilized or ingested by multiple routes. Each of these pathways come with their own set of microbiological risks and protection factors. Looking at these closely makes it relatively clear what kind of safety tests should be performed on finished products prior to use. It also makes it clear that most safety concerns are best addressed in the course of the actual production process itself.

Plant growth

Cannabis is grown under many different conditions, both indoors and outdoors. As with all agricultural products, it is exposed to an extremely wide range of microorganisms. However, the cannabinoids produced by the external glands of the plant have very well-documented antibacterial properties[15-21]. Living Cannabis plants do not support high levels of bacterial growth, and pathogenic bacteria are unlikely to be associated with living Cannabis plants. There is also some evidence for anti-fungal activity of certain cannabinoids[20], but fungal growth is not at all uncommon on Cannabis plants. Most of these mold and mildew species are plant pathogens, and not human ones; molds such as *Botrytis cinerea* may harm the Cannabis plant, but they are unlikely to harm humans.

Nonetheless, mold is perhaps the single most important quality issue in Cannabis production. Outdoor plants are exposed to a wide variety of fungal species. Indoor plants are exposed to less of these, and can potentially be kept cleaner. In practice, however, many indoor plants are exposed to inappropriate watering, humidity, fertilizer, or ventilation conditions. All of these can contribute to very high levels of mold.

Even under ideal conditions, it is possible that small numbers of cells or spores capable of causing human disease may be present on plant material from contact with air, soil, or water. If any of these species are capable of replicating aggressively either on dried plant material or upon contact with humans, they could theoretically prove to be a threat.

Processing.

Once plants are harvested they are trimmed, dried, and cured. These processes present significant opportunities for contamination. Harvesting and trimming are the stages at which there is the greatest level of human handling. Human pathogens can easily be transferred to the flower material at this stage. Workers should wash their hands frequently, and generally conform to the food safety rules that operate in commercial kitchens. Use of gloves during direct handling of the material should be mandatory.

Most Cannabis is dried and cured to a final water activity level of A_w 0.30 – 0.60 (unpublished data: OG Analytical, CannaSafe Analytics, AquaLab). This corresponds to moisture content values of between 2% and 13% [Fig.1]. Humidity and temperature need to be carefully controlled during this period in order to ensure that the moisture content of the plant material is lowered at a steady rate that balances the need to allow chlorophyll evaporation with the need to minimize

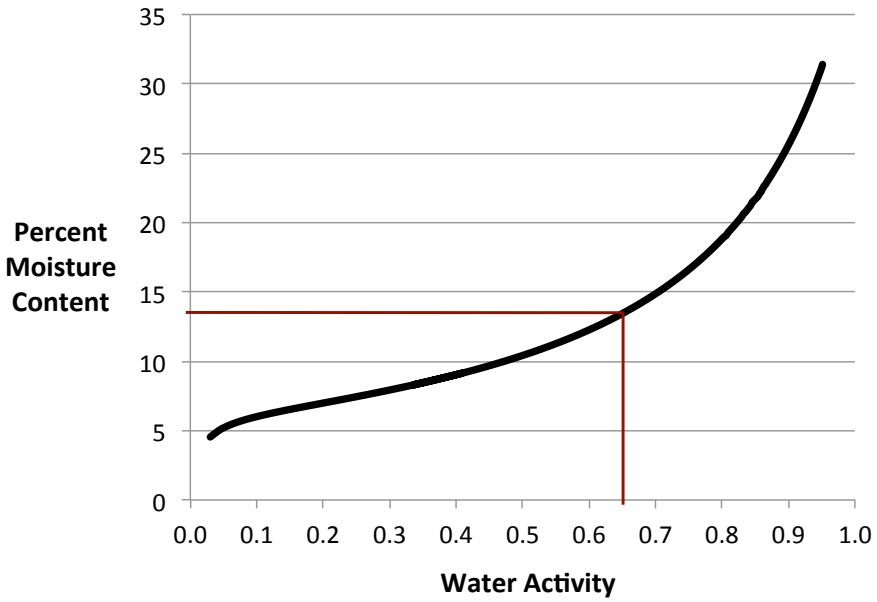

Fig. 1. Cannabis moisture sorption isotherm. Four independent isotherms were generated from unique samples of dried Cannabis flowers, and used as inputs to generate a generalized isotherm model for Cannabis. The curve shown represents average values; moisture content at A_W 0.65 will typically correspond to moisture content within two percentage points of 14%. Data was generated by AquaLab and CannaSafe Analytics using an AquaLab Vapor Sorption Analyzer.

overall moisture. If moisture levels are too high during this period, both mold and bacterial levels will rise above acceptable levels. If the drying process is done correctly, it kills the majority of the microorganisms that are present. Certain types of bacteria and fungi, however, are quite resistant to drying; even though they cannot grow at low water activity levels, they remain viable and can grow if conditions change.

Once cured, flower material can be smoked or inhaled by vaporization, but it is also frequently used to make extracts or concentrates that can themselves be smoked or vaporized, or added to products intended for oral ingestion. These edible products are extremely varied, but they typically all rely on the addition of a plant extract containing active cannabinoids. Simple approaches to extraction of these use butter or oil in which plant material is heated. The plant material is then removed and the butter or oil can be used for cooking. More complex methods use butane, hexane, CO_2, or other compounds as extraction solvents. These methods are becoming extremely popular, and they are now the prevalent form in which marijuana is used for the manufacture of edible products. They often utilize extremes of temperature and pressure, and they are unlikely to allow microbes to survive.

If the end product of the extraction process is intended for use as a food additive in Cannabis products, it is often subjected to an independent heating step. THC is found in plants in the acid form (THC-A), and is not psychoactive until it is converted through heating into decarboxylated THC[23,24]. The medicinally important (but non-psychoactive) cannabinoid CBD is likewise converted by heat from the CBD-A found in plants. This decarboxylation step is essentially a heat-kill step, and it contributes to the safety of Cannabis extracts and products made from them for the recreational market. The same decarboxylation process happens during smoking. In either case, the temperatures required are high enough to kill growing bacteria and fungi; however, they are not high enough to kill spores. Decarboxylation is a function of temperature and time, as shown in Fig. 2. Typical decarboxylation procedures use 120°C for 30min[22]. It is important to point out that decarboxylation is also a function of matrix viscosity and surface area. For instance, Cannabis extracts with high viscosity that are heated without stirring do not easily release the CO_2 byproduct of decarboxylation. These can require much higher temperatures or many more hours of treatment than other Cannabis products do.

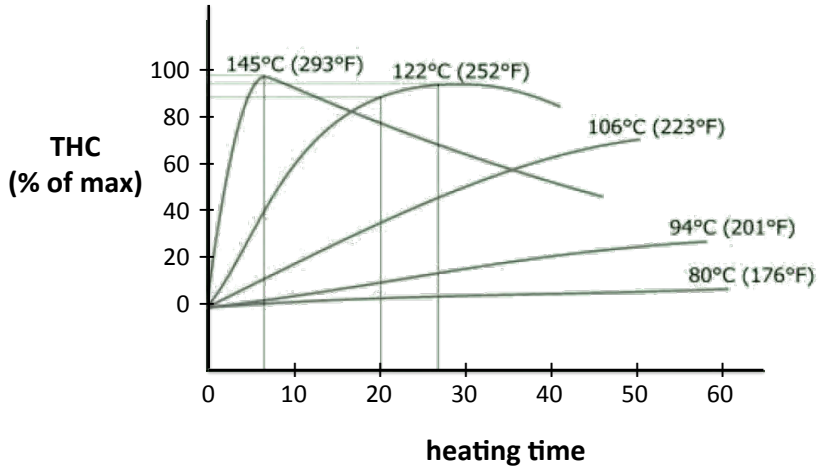

Fig. 2. Decarboxylation of THC-A to form THC. The curves shown indicate THC concentrations as a function of time, at various temperatures. THC values increase to their maximum as decarboxylation of THC-A into THC proceeds. They then begin to decrease as THC is converted into CBN (cannabinol). These data are from one particular matrix; many Cannabis extracts will have different dexarboxylation curves. Figure modified from Veress et al., (1990)[26].

Use

Cannabis is now commonly used in many different forms and by many different routes of administration. The primary methods are smoking of plant material or extracts; vaporization of plant material or extracts; and oral ingestion of extracts or foods made with extracts. Smoking or vaporization both involve heating to a temperature that will kill all non-spore microbial cells. Vaporization is a method of inhalation that uses lower temperatures than smoking in order to release volatile cannabinoids without burning solid plant material itself, but even these temperatures (typically a minimum of 160°C) are higher than microbial cells can survive.

Many edible products are either heated or made with previously heated extracts. However, there is a growing trend toward the use of medicinal Cannabis preparations that are *not* activated by decarboxylation. Some products are made using typical extraction procedures but without the heating step; others are made by direct use of fresh or dried plant material in, for instance, blender-prepared

shakes. Many medical marijuana patients report benefits from THC-A or CBD-A-containing products that they do not find with decarboxylated THC or CBD. Whether or not these medical benefits stem from THC-A, CBD-A, or other compounds, there is a potential advantage to patients, especially pediatric patients, if they can obtain them while avoiding intoxication from active THC. Fresh Cannabis products will have a different set of microbiological risks than cured and heated Cannabis will. Nonetheless, clear regulations should be developed for these uses as well. They are increasing in prevalence, and there is published data suggesting that THC-A has quite different immunomodulatory effects than THC does[25].

Non-ingested cannabinoid preparations are now common, such as topical creams and even transdermal patches. These present less microbiological risk because the skin is an effective barrier to infection.

Risk categories

Going through the stages of Cannabis growth, processing, and use makes it straightforward to identify the possible infection risks. Any danger would be due to a combination of factors across each of these stages. Pathogens would have to arrive on the plant during growing or processing, survive all processing and use steps, and then they – or their toxins – would have to be transferred to a human host in a way that allows them to cause disease.

Bacteria and fungi require moisture and medium-to-low temperatures for replication. The comparison with those agricultural products that are known to mediate infections is useful. Lettuce and Cantaloupe, for instance, are not known to have antibacterial properties, they are kept fresh and moist during processing, and they are ingested orally without any heating whatsoever. Still, infections from these sources remain quite rare, and individual events are extremely newsworthy. The rarity of these events is probably primarily due to the fact that human pathogens are not especially common on plants. Most lettuce or fruit-borne outbreaks of bacterial sickness have been due to human contamination of soil, or water, or processing surfaces.

Cannabis, in contrast, has inherent antibacterial properties, is dried well, and is usually then heated during processing or use. This makes it as safe as any agricultural product could possibly be. Nevertheless, these conditions don’t rule out all microbial threats.

These are the ones that remain:

* *Bacteria resistant to low water-activity.* Pathogenic bacteria that are extremely resistant to drying could potentially live on Cannabis, and be transferred to humans or to other items and then to humans. They would not survive the heat of smoking or decarboxylation, but they could nonetheless be carried into homes and come into contact with hosts through their presence on Cannabis. The only organism of concern in this category is *Salmonella*.

* *Fungal spores.* These are extraordinarily resistant to heat, and could survive the heat of smoking or decarboxylation. These are not known to cause disease through the oral route, but the spores of certain species in the genus *Aspergillus* can enter the lungs, germinate, and cause invasive lung disease in susceptible individuals.

* *Bacterial spores.* In theory, these could pose a danger just as fungal spores might. Bacterial spores could survive on plant material or in infused edible products, and enter the lung or stomach. However, there are no such spores that pose a threat under the conditions Cannabis is subject to. This will be discussed below in the section dealing with *Clostridium Botulinum*.

* *Toxins*. In theory, either bacterial or fungal toxins could be present on Cannabis because of the earlier presence of high levels of toxin-producing organisms. These could then be transferred to the lungs by smoking, or transferred into foods and delivered to the stomach. Alternatively, toxin-producing organisms could be present in food products and produce toxins there that remain a threat. We will deal with each of these possibilities below. In short, none of them are possible with Cannabis, because the conditions required for the high level of replication needed for toxin production are never available. In addition, the potential toxins of concern are all rapidly degraded rapidly by heat.

These are the categories that potential dangers could fall into. In the sections below we will cover each of the potential organisms or toxins that could mediate these threats. We have included only those that have plausible relevance, based on the public health histories of the food and agriculture industries. We know of no other microorganisms that should be of concern. Of course there are many other human pathogens we have not mentioned here, but they fall into the same categories as those that are safe or irrelevant.

Bacterial pathogens

Below is a discussion of the bacteria that could potentially be of concern on Cannabis. It is not exhaustive, as there are millions of bacterial species, thousands of which can potentially be human pathogens. These are just the species that are known to cause disease transmitted to humans from food or plant material, or those that have already been identified growing on Cannabis.

Listeria monocytogenes

Listeria can cause severe infection if ingested[26,27] and cases of Listeriosis have primarily been associated with contaminated foods. Cannabis products that are not eaten are therefore not a threat, but all food products should be handled so as to minimize the risk of Listeriosis.

Listeria is capable of growing at refrigeration temperatures[28-32]. It also forms very robust biofilms[33,34], so a common source of food contamination is work surfaces that have not been cleaned properly. Listeria is not dangerous in small amounts, and it is usually not dangerous in healthy people with no specific risk factors. It needs to be ingested in relatively high quantities to cause infection[35,36-39], and those particularly at risk are infants, adults older than 50, pregnant woman, and those with compromised immune systems[27,40-41].

Listeria cannot survive heating[42-45] and it requires high water activity levels in order to replicate[46,47]. Therefore dry products and heated or cooked products are all safe. *Listeria* will not grow on dried Cannabis flowers, and the decarboxylation process or the cooking process will kill any Listeria that may be present on Cannabis-infused food products. As with any food product, there is the danger for contamination after the cooking process and prior to consumption or packaging. Refrigeration does not remove this danger. It is critical that all producers of edible products follow good manufacturing practices, with special focus on keeping work-surfaces clean. High water activity foods that come into contact with unclean surfaces after cooking are at risk for Listeria. Low water activity foods are generally safe, and dried Cannabis is not at risk for *Listeria* contamination.

Escherischia coli

E. coli is a ubiquitous bacterial species that lives in the gut of many animals, including humans. It is generally not associated with disease, except for a subset of particular strains that produce a variety of toxins[48,49]. The most well-known of these is strain 0157:H7[50-52]. Outbreaks of this and other pathogenic *E. coli* strains[53] have typically been associated with contaminated meat products[54-56] or leafy greens[57-59]. Leafy green vegetables are a particular risk, as both soil and water can be contaminated with *E. coli* because of contact with fecal matter, and because these products are consumed raw. *E. coli* is killed by heating to temperatures higher than 160°F. Any meat, fruit, or vegetable product that is not cooked is a potential source of *E. coli* infection.

However, pathogenic toxin-producing strains are extremely rare[60]. In addition, they cannot grow at low water activity, or at refrigeration temperatures, and of course they are killed by high temperatures[61-63]. Therefore, a product such as Cannabis, that is both dried and then heated, is not a plausible vehicle for *E. coli* infection (Cannabis that is consumed fresh is an exception to this). Any food product may be infected with bacteria after cooking. If *E. coli* were to contaminate a Cannabis-infused food, it would be unlikely to have originated with the Cannabis. To be safe, all food products with high water activity should be kept refrigerated, and good hygiene practices should be followed by workers in production facilities.

Despite the fact that Cannabis is unlikely to present a special risk with regard to pathogenic *E. coli,* it is still possible that some amount of general non-pathogenic *E. coli* may be present, and this makes it potentially useful as an indicator test. Indicator tests will be discussed below.

Salmonella

Salmonella is a genus of bacteria of which there are only two known species: *S. enterica*, and *S. bongori.* There are many sub-species, or serovars, of *S. enterica*, but in general all types of *Salmonella* are considered pathogenic[64,65]. *Salmonella* is unlikely to be present on modern well-maintained Cannabis crops. It is also killed effectively by the temperatures of smoking or decarboxylation[66,67]. In addition, *Salmonella* is an intestinal pathogen, so the real danger of *Salmonella*

infection is always associated with ingestion of food products. For these to carry *Salmonella* that was originally associated with Cannabis, the *Salmonella* would have to survive both the extraction process and the heat of decarboxylation. Cannabis is no more likely than any other ingredient to serve as a vector for the introduction of *Salmonella* into a particular food product or a kitchen. If standard food safety guidelines are followed in kitchens producing Cannabis edibles, the vast majority of *Salmonella* infections can be avoided.

All of these factors taken together indicate that *Salmonella* is unlikely to be a problem on Cannabis. Nonetheless, *Salmonella* is unique in a number of ways that make it impossible to rule out as an issue. The first is that *Salmonella* is unusual in its ability to survive at extremely low water activity levels[68-70]. It cannot replicate under these conditions, but it can survive in a dormant state, and under the right circumstances it can be revived[69]. The second is that *Salmonella* is highly infectious. Unlike most bacterial pathogens it can initiate infection with doses potentially as low as a single cell[71].

In addition to these biological aspects, there is some sleight historical evidence for concern. In 1981 there was a *Salmonella* outbreak in four states that was traced to contaminated marijuana[72,73]. More recently, metagenomic sequencing data has detected small levels of *Salmonella* associated with the Cannabis root system (Jack Gilbert, unpublished data). The *Salmonella* identified in this way was a miniscule proportion of the total microbial load, it was not in the flowers or leaves, and this data has not yet been replicated. And the 1981 outbreak was very likely a result of extremely low-quality, high-moisture material simply serving as a vector to deliver *Salmonella* into people's homes. Nonetheless, both of these reports are cause for concern, simply because of the extraordinarily high infectiousness of *Salmonella*, and the general severity of *Salmonella*-induced disease, especially in the immunocompromised or elderly.

Salmonella is not uncommon in the environment, and can be found as a contaminant of both soil and water[74-77]. It is a potentially quite dangerous pathogen, it could be found on Cannabis, and it would survive the curing process. Were even small amounts to avoid a heat-kill step in a systematic way, they could cause an outbreak. For instance, it is possible that *Salmonella* could be transferred to Cannabis-infused edibles after inadequate heating for decarboxylation. Most smoked Cannabis should not pose a threat, however, it is still a common practice to smoke Cannabis cigarettes. This brings unheated plant material in close proximity to the mouth, and could lead to

infection.

All of these scenarios are unlikely, but they can't be dismissed. Properly handled material is very unlikely to pose a threat from *Salmonella*, and we believe state regulators should use their own judgment about whether it makes sense to incur the costs associated with *Salmonella* testing on Cannabis. Nonetheless, until further evidence argues otherwise, we recommend that Cannabis be tested for *Salmonella*.

Thermophilic actinomycetes

Actinomycetes are a very large group of bacteria, containing many thousands of species. Some of these are capable of forming branching hyphal structures that can grow into biofilms resembling fungal mycelia[78]. Thermophilic ones are capable of growing at high temperatures and are dominant in composting plant material[79,80]. They were found on Cannabis in a report from 1983[81], and although not mentioned again in this context since then, they made it onto the initial list of required tests for Cannabis in Colorado in 2013. This paper actually found three species thought to be in this category: *Thermoactinomyces vulgaris, Thermoactinomyces candidus*, and *Micropolyspora faeni. T. Candidus* was later identified as being the same species as *T. vulgaris*[82]. *M. faeni* was later reclassified twice, ultimately becoming known as *Saccharopolyspora rectivirgula*[83]*. T.vulgaris* and *S. rectivirgula* (along with *Aspergillus* species) are common causes of the allergic reaction known variously as farmer's lung, hypersensitivity pneumonitis, or extrinsic allergic alveolitis (EAE)[84,85].

We are not aware of any data which indicates whether these bacteria (or their antigens) can survive the temperatures of burning or vaporization and still cause allergic reactions. But there is no correlation in the literature between smoking cannabis and EAE. Neither is there any known correlation between these allergic reactions and tobacco smoking – although such reactions have been noted among workers in the tobacco processing industry who are exposed to spores directly[86].

Thermophilic actinomycetes are ubiquitous in soil. They can multiply to very high levels in composting plant material, and chronic direct exposure at these levels can occasionally cause these allergic

hypersensitivity reactions. However, the fact that these species grew on agar plates exposed to Cannabis (as with *Penicillium* and *Mucor*) is primarily an indication of the particular nutrient characteristics of the plates that were used in this 1983 study. If anything, what is notable is that so few species grew on these plates, when today we understand that many thousands of different species are present on any plant or soil sample[87-90].

Actinomycetes are ubiquitous, and yet unlikely to be numerous on properly dried Cannabis. They are not human pathogens, nor are they likely to cause allergic hypersensitivity reactions upon smoking or ingesting. There is no need to test for them on Cannabis.

Pseudomonas

Pseudomonas is a large genus of gram-negative bacteria with an extremely wide range of metabolic capabilities and an accordingly wide range of habitats[91,92]. Pseudomonas has been detected on Commercially grown medical Cannabis (Darryl Hudson, personal communication) and there has been some level of concern about this because of the potential for pathogenic *Pseudomonas* to cause infection in immunocompromised individuals[93-95]. However, there is only one common human pathogen in the Pseudomonas genus, and it is not likely to be dangerous even if it was found on Cannabis.

Three species of *Pseuodomonas* are potentially relevant in the cultivation of Cannabis. The first is *P. syringae*, a common plant pathogen that is not pathogenic to humans. Although this species is capable of harming crops, it is is not a safety concern. The second is *P. fluorescens*, which is a "biocontrol" organism with the ability to promote plant growth through an unknown mechanism. This species is commercially available, and may be found on Cannabis plants to which it was deliberately added. Such biocontrols are generally a positive way of handling horticultural problems, especially in comparison to chemical pesticides. *P. fluorescens* is not a danger to humans.

The third relevant species is *P. aeruginosa*. This is the human pathogen in the genus, and it is one of the most common causes of hospital-acquired bacterial infection[96-98]. It is generally not able to infect healthy individuals, but it can cause infections in the immunocompromised or those with chronic pulmonary diseases such as cystic fibrosis[93,99-103]. It has an extremely broad range of potential

habitats, and is essentially ubiquitous in the environment[104-107]. It is found in water, soil, on many plants, and can colonize many different types of surface[104,108-111]. It is responsible for water-born infections of the ear[112-114], eye infections related to contact lenses[115-117], and many different types of internal infections subsequent to wounds or other injuries[118,119].

As with several other human pathogens, *P. aeruginosa* is potentially dangerous, but it is also an organism that we are generally in constant contact with. Infection only takes place when an inoculum is high, immunological protection is low, and a specific route of delivery to a susceptible site in the body is provided[120-122]. Cannabis does not provide a delivery method that will allow it to initiate infections. The oral dose required to initiate infection is extremely large[104,123]. The inhalation dose is thought to be lower[124]., but *P. aeruginosa* is highly sensitive to both dessication and heat[125-129]. As with the majority of bacterial species, it will not survive the drying process that occurs when Cannabis is cured, and it will not survive the heat of smoking or decarboxylation treatment.

Fungal pathogens

Mold, mildew and yeast are all types of fungi. Mold in particular is very common on agricultural products. Certain types can grow on live plants; others, termed saprophytes, generally grow on dead plant material. Cannabis is host to many mold species of both types. The molds that are common on living Cannabis, such as *Botrytis cinerea*, are plant pathogens, not human ones[130]. Non-pathogenic molds can be a source of allergic hypersensitivity reactions[131-133], but there is no evidence associating such reactions with smoking. As discussed above, a number of pathogenic mold species have been isolated from Cannabis kept under extremely poor conditions[11,81]. Spores of these species are ubiquitous, and Cannabis presents no special risk for fungal infections caused by them. However, certain molds of the genus *Aspergillus* do present a risk.

Aspergillus

Aspergillus is a mold that produces extremely hardy spores, and is capable of replication at much lower water activity levels than most organisms[134-136]. It is also ubiquitous; *Aspergillus* spores are thought

to exist in soil and on plants essentially everywhere[137-139]. Gardeners and farmers in particular are believed to breathe in thousands of spores every day[140-142]. Under normal conditions, the human immune system removes these from the lungs[143-146]. In the immunocompromised, however, certain *Aspergillus* species can cause invasive lung disease[147-156]. Invasive pulmonary aspergillosis is extremely hard to diagnose and to treat, and the mortality rate is quite high[157-162]. In addition, there is a known clinical association between Cannabis smoking and pulmonary aspergillosis. Cannabis smoking is considered a clear risk factor for this disease, and there are many cases on record[163-170]. It appears likely that the spores can survive the heat of smoking and are mobilized by the smoking process and transferred to the lungs. In the absence of a healthy immune system, the spores can germinate and colonize the lungs.

This is particularly significant in the case of the modern medical marijuana industry. Pulmonary aspergillosis is the one serious documented microbiological safety threat to Cannabis smokers. It usually takes hold only in the immunocompromised, but many medical marijuana patients have diseases – such as cancer or HIV infection – that result in damaged immune systems. In addition there is thought to be a dose effect[171,172]. Plant material that was improperly dried or handled and has higher mold levels could potentially present a higher risk.

Aspergillus is ubiquitous, but the majority of *Aspergillus* species are not pathogenic. There are hundreds of species in this genus, and most of them are harmless. The species thought to be responsible for the vast majority of cases of human aspergillosis are these: *A. fumigatus, A. flavus, A. terreus*, *A. niger*, and potentially *A. nidulans*[147,162,173,174]. *A. fumigatus* alone is likely responsible for about 75% of *Aspergillus* infections in the U.S[175].

All four of these species should be tested for. Samples that test positive for any of them should be returned to the producer. Returned samples cannot easily be sterilized because of the hardiness of fungal spores, and they should not be sold. However, they can reasonably be used for concentrate production destined for edibles.

Cannabis users should know the risks involved in smoking a substance that can contain viable *Aspergillus* spores. Those who are immunocompromised should be counseled to avoid smoking in general. Edible cannabis products are now widely available and will be safer for this population, as *Aspergillus* spores generally do not cause

disease when ingested orally.

It is important for legislators to understand that no data is available on the environmental burden of *Aspergillus* on Cannabis. When this data becomes available it may turn out that pathogenic *Aspergillus* species are quite rare on Cannabis. The opposite is more likely, however. It may be the case that *A. fumigatus*, in particular, is so common that all Cannabis samples (at least outdoor-grown varieties) contain some level of it. If this is the case, it will NOT make sense to require that all Cannabis be tested for *Aspergillus*. Healthy people have extremely high innate immunity to *Aspergillus*[176], and there is no advantage in testing for ubiquitous organisms.

However, choosing not to test for this pathogen could only be done in parallel with a concerted public health education campaign to alert immunocompromised patients to the danger of Cannabis-mediated Aspergillosis. There may also be some middle ground in which it would be reasonable to identify a threshold below which some *A. fumigatus* is acceptable if samples are clearly labeled with testing results. Nonetheless, given the information that is now available, we strongly recommend that all Cannabis be tested for *A. fumigatus, A. flavus, A. terreus, and A. niger,* and failed if positive for any of these.

Penicillium

Penicillium is a genus of fungal mold species, and it has been isolated from Cannabis plants. It is ubiquitous on plants and in soil, and it is fast-growing and extremely likely to predominate on the agar culture plates used for fungal culture in the 1980s. Although one *Penicillium* variety is an opportunistic pathogen of immunocompromised HIV patients in Southeast Asia[177,178], except in very rare cases[179,180] the genus is otherwise not a cause of human disease.

Mucor

This is a large genus of fungi containing over 3000 separate species[181,182]. As with *Penicillium*, they are ubiquitous, fast-growing, and very easy to recover on culture plates[183,184]. A very small number of these can cause human disease. This disease, known as mucormycosis, is extremely rare, and typically presents in non-immunocompromised patients only in cases where the spores are introduced to the body through "traumatic inoculation"[185-187].

Pulmonary forms of mucormycosis are know to occur, but usually only in patients with underlying hematological malignancy[188-191], and these are not associated with increased inhalation exposure.

Botrytis cinerea

This is one of the most common fungal plant pathogens. It infects many different crops, but is particularly an issue with wine grapes[192-195] (where it is on occasion a positive influence) and Cannabis (where it is not). It does not infect humans, and although allergic hypersensitivity reactions to it have been described, they are only two reports of it in the existing scientific literature[196,197].

Microbial toxins

Aflatoxins

Aflatoxins are a variety of mycotoxin produced mainly by two species of *Aspergillus (A. flavus,* and *A. parasiticus)*[198,199]. Because *Aspergillus* is ubiquitous, aflatoxins are as well, and many industries have set baseline levels for acceptable amounts of aflatoxin contamination[200-203]. However, the conditions necessary for the production of significant levels of aflatoxin are not present on Cannabis.

In order for aflatoxin production to occur, *Aspergillus* must initiate a successful colonization of some substrate that supports hyphae production and robust replication[204]. *Aspergillus* is a saprophyte, meaning it commonly grows on dead and decomposing plant matter[205-207]. It can also grow on living plants, but it requires high levels of oils and other nutrients for robust growth and aflatoxin production[208-210]. The agricultural crops capable of fulfilling these conditions are high-oil-content seeds, and certain grains and nuts[208]. *Aspergillus* replication on Cannabis would be possible only on extremely moldy post-harvest plant material, or on the seeds themselves. Because Cannabis flowers sold in dispensaries today are produced entirely from un-fertilized female plants that do not produce seeds, this is not a concern. In addition, even with permissive nutrient sources, aflatoxin production is halted at low water activity levels[211-213]. This is the case as water activity approaches A_w 0.9. Cured Cannabis is much dryer than this, typically under A_w 0.6.

Data do exist that could be interpreted to indicate a risk posed by aflatoxins on Cannabis. For instance, *Aspergillus flavus* is extremely widespread in soil, and some plants that cannot support *Aspergillus* growth are still capable of aflatoxin uptake from the environment[214]. There is no evidence that this is likely to lead to aflatoxin levels above established international exposure thresholds, and there is no evidence that it takes place in Cannabis. Another possible concern is that the heat applied to Cannabis during smoking or decarboxylation would not remove all aflatoxins. The aflatoxin molecule is somewhat heat-labile; it is degraded by exposure to heat levels above 160°C[215,216]. But decarboxylation and smoking temperatures are not always this high, and though they would lead to some degradation of aflatoxin, it would not be complete. Finally, Cannabis seeds have high oil content, and they would not be an unusual host for *Aspergillus*. There is a reasonable concern that Aflatoxins may be present in hemp seed products. It is also true that the hermaphroditic tendency of the Cannabis plant sometimes leads to the occasional seed in commercially sold Cannabis flowers, and these could potentially be colonized be *Aspergillus*.

Taken together, these concerns do not warrant batch testing of all commercial Cannabis for aflatoxins. The presence of detectable levels is highly unlikely, and after many decades of popular use, aflatoxin poisoning has never been linked to Cannabis use. Aflatoxins have been mentioned as a concern with respect to Cannabis, because aflatoxins do contaminate many other food products, and because *Aspergillus* itself is clearly a concern. But in this case it is invasive fungal disease that is a threat, rather than aflatoxin poisoning. Only one paper in the existing literature describes the isolation of aflatoxin from Cannabis[217]. In this study, the authors themselves added large amounts of *Aspergillus* flavus and *Aspergillus* parasiticus to Cannabis confiscated by police. They mixed the Cannabis with the *Aspergillus* in large amounts of water, and assessed aflatoxin production 14 days later. They then reported levels of aflatoxin production that were extremely low compared to other growth substrates. It is worth pointing out that cannabinoids have been found to have strong antifungal properties[20,218], and that the Cannabis used in this 1977 paper had a THC content of 1.5%.

Botulinum toxin

Clostridium botulinum is a spore-forming bacteria that produces an

extremely dangerous toxin[219]. The possibility of poisoning by botulinum toxin has been raised with regard to the production of Cannabis-infused edible products, and the Denver Department of Public Health has posted warnings on this subject. The reason for this concern is that several cases of botulinum poisoning in this country have been associated with oils infused with garlic, vegetables, or herbs[220-223], and many Cannabis products are made by first producing Cannabis-infused oil or butter.

C. botulinum is an anaerobic organism, and will only survive in environments where oxygen is absent[224]. For this reason, most cases of botulinum poisoning have been associated with infused-oil products, or with mishandled canned products. In both cases, spores found naturally in the environment or on agricultural products are delivered to an anaerobic environment where they can germinate, grow, and produce toxin. Moreover, *C. botulinum* can tolerate an extremely wide range of temperatures. Its spores are highly heat-resistant, and it can also grow, albeit slowly, at refrigeration temperatures. Many normal cooking processes do not kill the spores, but heating to 121°C for three minutes will do so[225-227]. The toxin itself is heat-labile, and can be destroyed by heating to just 85C for 5 minutes[228,229].

Most importantly, *C. botulinum* requires water activity of A_w 0.94 or higher for growth[230,231]. It cannot multiply on dry material. The reason why infused oils have been able to support growth is because adding fresh garlic or herbs or vegetables to oil creates a local region of very high water activity, in the center of an anaerobic environment. Botulinum poisoning has not been associated with dried herbal products, or oils infused with dried herbs. *C. botulinum* spores probably exist in many places, but they only replicate to the level needed to produce toxin under very particular conditions. The key to avoiding botulinum poisoning is to avoid adding products with high water activity to anaerobic media or to sealed containers. In the cases where this happens, such as with canning, there needs to be a "botulinum cook" step of heating to 121°C for three minutes, and/or products need to be kept refrigerated and discarded after several weeks[224,232].

Foods infused with Cannabis extracts made from cured Cannabis do not present a risk for botulinum poisoning. Most Cannabis foods now use use concentrates from hydrocarbon or CO2 extraction processes rather than oil or butter infusion. Those that do use infused oils are not at greater risk, assuming the plant material is properly dried. Once a food product is made, it can always be contaminated with any

organism. But *C. botulinum* will not replicate on a product with low or medium water activity. As with any and all food products, anything that has high water activity should be refrigerated, and discarded after several weeks. In the cases where oil or butter are made with raw Cannabis, these should be treated with special care. If the goal is to avoid decarboxylation, then the "botulinum cook" step is not feasible. Such products can still be safe, provided they are refrigerated and consumed soon after preparation.

Indicator tests

The discussion in the previous sections concerns identification of organisms that might act as human pathogens. However, a standard practice in microbiology safety testing in the food industry is the use of "indicator tests"[3]. These are tests for organisms that are not themselves pathogenic, but can still provide useful information[2-4]. In some cases, they can indicate a higher likelihood of the presence of pathogens, perhaps leading towards more focused testing. In other cases, indicator tests serve as "quality tests" and provide indirect information about the cleanliness of the production process.

Most testing for pathogens in the food-safety industry does not target end products. Testing programs are typically present in order to evaluate production processes and facilities. Even when limits are specified for specific organisms in an end product, this is still understood to be a way of evaluating the process itself, and is not applied to every single batch of a product. This is especially true of indicator tests; they were not designed to be applied to final batches of a product. Their value lies in the general information they can provide about production practices, rather than about the safety of any particular batch of product. Modern food safety practices rely on HACCP (Hazard Analysis and Critical Control Point) programs as a means of ensuring safe production environments. Microbial testing is used to develop data to guide HACCP programs, and in some cases to verify implementation[233,234].

Nonetheless, states with legal Cannabis programs are committed to a higher level of regulatory safety controls than are applied to other products. This includes end-point testing for pathogens that may be present; it may be reasonable to include indicator tests as well, if they can provide actionable information. It is critical, however, to avoid the mistake of requiring an entire industry to perform uninformative tests

on every single batch of its products.

Bacterial indicator tests

The tests required for Cannabis products in Washington State included a series of bacterial indicator tests of increasing specificity.

These were:

Total aerobic bacteria
Bile-resistant gram-negative bacteria
Total coliforms

Total aerobic plate counts are a very common indicator test; they are a quality test, and frequently have results that correspond to millions or tens of millions of bacterial cells on the sample. They are thought provide some general sense of the cleanliness of production or processing. Bile-resistant gram-negative bacteria is a category that was in use many decades ago and is no longer used in food-safety testing. The purpose of this category was to define a group of bacteria that would include the majority of gut-borne pathogens. Several other indicator categories have since gained favor for the purpose of showing the potential for fecal contamination. These include *Enterobacteriaceae*, total coliforms, fecal coliforms, thermotolerant coliforms, and generic *E. coli*. The majority of these tests have fallen out of favor, as they do not accurately represent the threat of fecal contamination[5,6]. Many of them include species that are harmless and that can arise from other sources. The test most commonly recommended now as an accurate proxy for fecal contamination is general *E. Coli*[235,236]. This genus of bacteria is extremely common in the mammalian digestive system, and relatively rare elsewhere. Fecal contamination during the production of Cannabis could arise with workers washing their hands incompletely, or from contaminated soil or water. None of these cases would necessarily mean that the Cannabis was dangerous to use. However, it could indicate the possible presence of enteric pathogens, and it is likely to reflect a problem in the production process.

Fungal indicator tests

Mold is the most common type of microbial growth on Cannabis. It is a constant source of practical and financial difficulty for Cannabis

growers, and most states that require Cannabis testing at this time include the classical microbiological test known as "total yeast and mold". These assays are plate or film-based culture assays that are intended as pan-fungal broad-spectrum indicator tests. The majority of the molds that grow on these plates will be common plant pathogens, and are highly unlikely to cause human disease. As with total bacterial tests, tests for "total yeast and mold" are essentially a quality test. They are unlikely to serve as a good indicator for the presence of pathogens. In fact, the typical plate assays used to assess total yeast and mold levels are able to support the growth of only a very tiny percentage of the fungal species common in the environment, and they show poor correlation with eachother[237-240].

There are two rationales for requiring such a test for Cannabis. The first is that since mold is so common on Cannabis, and high levels are likely as a result of many different environmental and processing factors (harvest timing, seasonal rain levels, curing processes, cross-contamination, etc), it makes sense to include a total yeast and mold test as a general quality indicator. The other rationale is that -- even though such tests do not serve as indicators for the potential presence of pathogens – mold is a potential cause of irritation and allergic hypersensitivity reactions.

The broad spectrum of allergic reactions to inhaled antigens are usually grouped under the terms hypersensitivity pneumonitis or extrinsic allergic alveolitis. These are IgE-mediated inflammatory reactions to an extremely wide variety of antigens including inorganic molecules, avian proteins, bacterial endotoxins, and bacterial and fungal spores. Applying the total yeast and mold test to Cannabis, however, is extremely unlikely to minimize the number of allergic hypersensitivity reactions among Cannabis smokers.

There are many reasons for this. The most common causes of allergic hypersensitivity are bacterial or non-microbial, and therefore won't be detected on fungal culture plates[241,242]. The most common causes that *are* fungal are *Aspergillus* species[243-245], for which total yeast and mold tests are not good quantitative assays, and which need to be tested for on Cannabis independently in any case. Botrytis is probably the most common mold on Cannabis plants, and although there are reports of hypersensitivity reactions to it, these are rare, and usually involve extremely high exposure levels[197,246]. In addition, among tobacco smokers there is no evidence of increased allergic reactions to microbial antigens[81,247-249], which argues that such antigens are either degraded by smoking, or not mobilized by it. Combined with the fact

that "total yeast and mold" tests can culture only a small percentage of fungal species, all the above evidence argues against using such a test to prevent hypersensitivity reactions.

This test could still be used as a general quality indicator. Extremely high levels of mold on Cannabis flowers are generally considered an indication of poor curing or handling practices. However, relatively high levels are present on most Cannabis. Some states have chosen 10^4 CFU/gram as the total yeast and mold cutoff for Cannabis. This is a value that causes many apparently acceptable samples to fail. 10^5 CFU/gram may be a more reasonable cutoff, though this is a level at which mold is typically visible by eye (and certainly by microscopy). To our thinking, it is difficult to justify the resource and pricing impacts imposed by a test of this nature, when the benefits are unclear, the appropriate cutoff is unknown, and visual inspection is a viable alternative.

Recommendations

In comparison to most agricultural products, Cannabis is exceptionally safe. Nonetheless, the authors of this white paper are in agreement that Cannabis can pose a microbiological safety hazard if the proper regulatory controls are not in place. Primarily this is because it is a very large industry, and also because of the particular risk for aspergillosis associated with inhalation.

Several safeguards are inherently present in the processing and use stages for most Cannabis products, and these can be monitored using straightforward methods. Other safety measures can be implemented by proper testing procedures. These include statistical sampling techniques, tests for relevant microorganisms, and proper assay design and validation. Recommendations for each of these safety measures are outlined below. If they are followed, Cannabis will be a much safer product (at least from a microbiological perspective) than any of the produce we buy in the grocery store.

1. Water activity can be used as a marker for overall microbial levels. Plant material with high water activity will support microbial growth. Because the drying step is one piece of insurance against microbial dangers associated with Cannabis, it makes sense to require that this step be complete. The majority of commercially sold Cannabis is dried to water activity levels that are below the minimum threshold

for any type of microbial replication. Samples that are above this minimum level (0.6 A_W) are at slightly higher risk. However, very few bacterial or fungal species can replicate between A_W 0.6 A_W 0.7. Above A_W 0.7, microbial growth begins to be more possible. We recommend that all curing processes aim to produce flower material that is under Aw 0.6, and that flower material above A_W 0.65 be returned to producers for further curing.

3. Fresh Cannabis will require a different set of microbiological guidelines. There has been a rise in the popularity of preparations of Cannabis that are either not dried or not heated, or both. Ingesting raw Cannabis is analogous to ingesting lettuce - it is high water activity and consumed without heating. It's not accurate to say that eating raw plants is dangerous. If Americans did so more often, we'd be much healthier. Nonetheless, it increases the small chance of certain types of microbial infection. In the context of large distribution pipelines for commercial agricultural products, there is an elevated risk for certain bacterial infections that is not present with dried or cooked foods. Products made from Cannabis that is cured but not heated will occupy a middle ground. If they are also subjected to hydrocarbon or CO_2 extraction, they will be quite safe. However, products made from fresh raw Cannabis for commercial purposes should be subject to increased microbiological surveillance for Pseudomonas aeruginosa, Clostridium botulinum, and toxigenic E. coli.

3. Edible Cannabis products should be regulated by local health departments. Cannabis food products should not be subject to end-product testing for microbiological contamination. The commercial facilities making these products should follow modern HAACP guidelines and be inspected and regulated by local and state health departments just as all other commercial food production facilities are.

There are many reasons for this recommendation. Food products always pose some risk for the spread of food-born illness, and the knowledge about how to mitigate these risks is now quite advanced in this country. It therefore makes sense to follow the best-practices guidelines that already exist for the food industry. These are extensive, and they are based on continuous monitoring of production processes and environments themselves, rather than end products.

Cannabis food products are as likely to become contaminated as any other processed or prepared commercial food product. But because of

its unique attributes, *Cannabis is the least likely component to be the source of contamination in any food product*. Cannabis is present in foods as an extract of the plant material. This plant material is dried to a safe level before extraction. And then either during or after extraction it is usually subject to a decarboxylation process that serves as a heat-kill step. The vast majority of the extraction processes are themselves sterilizing. Once these extracts are added to food, the food can always be mishandled or subject to "temperature abuse", which raises the chances of contamination. But these are factors facing all foods, and the only pathogen of real concern on Cannabis (*Aspergillus*) is not infectious by the oral route. Cannabis food products should be regulated as all food products are, which means that the facility must prepare and follow an adequate HAACP safety plan, and the local or state health department must be vigilant about inspections and standards.

The Denver Department of Public Health (DDPH) serves as an excellent model for how to carry out this approach. The regulators from the DDPH have been rigorous about monitoring the supply chain of Cannabis-infused edibles in Denver. They have treated the legalization of these products there as simply an increase in the number of small and medium-sized commercial kitchens within its jurisdiction, and they have held all of them to strict standards. In the cases where food products were prepared in jurisdictions not subject to adequate public health oversight, the DDPH did not allow these products to be sold within Denver city limits.

4. Cannabis should be tested for four species of *Aspergillus*: *A. flavus, A. fumigatus, A. niger,* and *A. terreus.* Together these species are responsible for the vast majority of cases of invasive pulmonary aspergillosis, and they are the only pathogens that represent a clear and certain danger on Cannabis.

5. Cannabis should be tested for total generic *E. Coli*. Samples with levels above 100 CFU/gram should be rejected. This is the one indicator test that we recommend. Detection of significant levels of E.Coli are strong evidence of problems during growing or processing, including contaminated soil or wate, or improper handling. E. Coli is now accepted to be the optimal indicator organism for the identification of possible fecal contamination. Were pathogenic bacteria such as E.Coli or *Salmonella* to be present, they would likely have arrive through this type of pathway, therefore samples positive for

E.Coli are both higher risk and indicative of general production problems that need to be addressed.

E. Coli is usually not pathogenic, and many food-safety protocols do not require it to be entirely absent. A general guideline for E. Coli testing, and one which fits well with existing Cannabis testing data, is that no product should have over 100 CFU/gram (or equivalent) of generic E. Coli.

6. Cannabis should be tested for *Salmonella*. Samples with detectable Salmonella should be rejected. The odds of *Salmonella* infection from Cannabis are very low. Nonetheless, it is the one bacterial pathogen that poses a potential threat to Cannabis smokers. There is precedent for *Salmonella* association with Cannabis in both this early epidemic, and in very recent microbial sequencing data. It is highly infectious and can cause disease with as low a dose as one single cell. It is hardy and highly resistant to dessication. And it has a mortality rate that is significant, and significantly higher in older or immunocompromised patients that are likely to be exposed through the use of medical Cannabis.

All Cannabis flower material should be tested for *Salmonella*, with close attention paid to the statistical sampling methods discussed below. Batches with any detectable *Salmonella* should be failed.

7. There is no need to test *Cannabis for Pseudomonas aeruginosa, Listeria,* toxigenic *E. Coli* (e.g., H7:0157), or other bacterial pathogens besides *Salmonella*. Cannabis is not a potential delivery vehicle for these organisms, or for most bacterial pathogens. Because it is both dried and heated before use, it has undergone two highly effective sterilization steps, and none of these pathogens can survive both of these. All of them will die if exposed to the heat of smoking or decarboxylation, and all of them will generally be rendered noninfectious by the curing process.

This does not mean that mis-handled or improperly cured Cannabis could not be a vehicle for these organisms. As with any agricultural or food product, it can be a source of increased hazard if it is maintained at high water activity levels, if typical decontamination steps are not performed, or if it is consumed fresh.

8. There is no need to test Cannabis for "total yeast and mold". Total yeast and mold tests detect only a small fraction of the fungal species in the environment, and do not correlate with the presence of pathogenic species. The only pathogenic mold species on Cannabis are types of *Aspergillus* that must be tested for separately in any case. Molds can potentially be a cause of allergic hypersensitivity reactions, but there is no evidence that these are mediated by smoking. Molds can also be a source of plant spoilage, but these processes can be monitored appropriately by testing for water activity levels, and by visual or microscopic inspection.

9. There is no need to test Cannabis for aflatoxins. These would be at least partly degraded by the heat of smoking or decarboxylation, if they were present. But seedless Cannabis plants are not capable of supporting aflatoxin production, because they lack the high oil content necessary for *A. flavus* replication.

10. Statistical sampling procedures must be used for microbial testing. It is common practice in many Cannabis testing labs to accept individual 1g flower samples from growers or dispensaries. This is a practice guaranteed to make test results highly misleading. Pathogens are not spread evenly on surfaces, but instead cluster in local colonies. THC testing faces a similar issue: levels can vary by as much as two-fold across different regions of an individual plant. Statistical sampling techniques performed by trained lab personnel will largely solve both of these problems.

The entire batch to be tested must be present, and multiple sample increments should be collected using a statistically random sampling procedure. These must be collected by laboratory personnel, not by employees of the grower or retailer. Batch sizes are defined differently from state to state, and even within a single state there are many situations that can lead to a variety of batch sizes. Microbiological testing is not meaningful if the fraction of the total volume sampled is not identical between tests. Therefore it is essential that testing procedures require a set quantity of Cannabis to be sampled *per pound*, regardless of the total batch size.

We recommend that 5g per pound be sampled from every batch of Cannabis, in 5 individual, randomly chosen one-gram increments. All of these sub-samples should be combined together for the entire batch (for instance, a 5lb batch would require 25g of total sample material).

The combined sample must be thoroughly homogenized, and the appropriate volume removed for the performance of each assay.

Statistical sampling procedures are detailed in the protocols found in ISO 7002:1986 and ISO 4874:2000, and all laboratories should have published Standard Operating Procedures modeled after these. We further recommend that batch sizes be constrained to 5 or 6 pounds. Smaller batch sizes lead to testing prices that are overly burdensome to producers, whereas larger batch sizes lower the detection thresholds of certain assays.

11. Cannabis extracts and concentrates require different types of microbial screening. The process of extracting cannabinoids with hydrocarbon solvents (butane, hexane, etc.) is likely to be sterilizing. The same is true for both supercritical and subcritical CO_2. More data is needed to prove that this is the case, but the temperatures and pressures involved make it likely. Alcohol extracts are sterilizing as well, if they are made with high-proof alcohol. This is not because of temperature or pressure, but because most microorganisms cannot survive in alcohol. The only real possible danger in all of these cases is that spores may survive the extraction process, in which case *Aspergillus* testing would be needed on such extracts if they were destined for smoking or vaporization, but not if they were intended for infusion into edible products.

Many concentration processes do not use solvents, but use water or mechanical force to remove and concentrate the cannabinoid-rich external trichomes on Cannabis flowers and leaves. These products are all essentially varieties of hashish, and in general they are low water activity. In some cases, however, they can have enough moisture content to be support fungal or bacterial growth. More data is needed on these products as well; until then they should be screened exactly as dry Cannabis flowers are screened.

Summary of Recommendations

1. Water activity can be used as a marker for overall microbial levels: Cannabis with water activity levels above A_w *0.65 should be returned to producers.*

2. Fresh Cannabis requires additional testing, which should include Pseudomonas aeruginosa, Clostridium botulinum*, and toxigenic* E. coli*.*

3. Edible Cannabis products should be regulated by local health departments. They carry the same microbiological risks as any food product, and heated Cannabis extracts do not increase this risk..

4. Cannabis should be tested for four species of Aspergillus: Aspergillus flavus, Aspergillus fumigatus, Aspergillus Niger*, and* Aspergillus terreus.

5. Cannabis should be tested for total generic E.Coli*. Samples with levels above 100 CFU/gram should be rejected.*

6. Cannabis should be tested for Salmonella*: Samples with detectable* Salmonella *should be rejected.*

7. There is no need to test cured Cannabis for Pseudomonas aeruginosa, Listeria*, toxigenic* E. Coli *(e.g., H7:0157), or other bacterial pathogens besides* Salmonella*.*

8. There is no need to test Cannabis for "total yeast and mold".

9. There is no need to test Cannabis for aflatoxins.

10. Statistical sampling procedures must be used for microbial testing. A total of at least 5 grams randomly distributed throughout each pound of flower material must be collected. These subsamples for the entire batch should then be combined, thoroughly homogenized, and the appropriate volume of this mixture utilized for each assay. Batch sizes should be 5-6 lbs.

11. Cannabis extracts made with hydrocarbon solvents, CO_2*, or alcohol should be tested for Aspergillus if they are intended for direct inhalation. They do not need microbial screening prior to use in edible products. Extracts made with water or without solvents should be screened for the same microbes as cured Cannabis flowers: four Aspergillus species, generic E. coli, and generic Salmonella.*

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