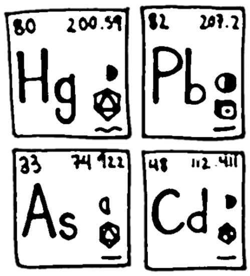
WHITE PAPER

The Importance of Measuring Heavy Metal Contaminants in Cannabis and Hemp *



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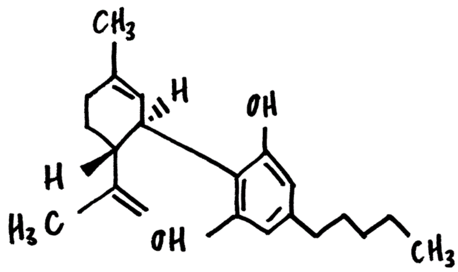
Introduction

The cannabis and hemp industry is moving at such an alarming rate that the analytical testing community is struggling to keep up with it. It is estimated that the demand for medicinal and adult recreational cannabis-based products, containing tetrahydrocannabinol (THC) and cannabidiol (CBD) compounds will exceed $25 billion in the US by 2025. However, because the US Food and Drug Administration (FDA) has only been involved in this process when an investigational new drug has been submitted to conduct human clinical trials, regulating the industry to make sure products are safe for human consumption has been left to individual states. In addition, CBD-only products, which are dominating today's marketplace, are, for all intents and purposes, unregulated by the federal government at this time.

Unfortunately, many of the states where it is legal do not have the necessary experience and background to fully-understand all the safety, quality, and toxicological issues regarding the cultivation and production of cannabis and hemp products on the market today. Besides the need to characterize its potency (CBD and THC content), one of the most important contaminants to measure is the level of heavy metals, because cannabis and hemp will avidly accumulate trace elements from the growing medium, the soil, fertilizers, and even the metallic equipment used during the preparation and processing of the various concentrates and oils. For that reason, it's critically important to monitor heavy metals in cannabis and hemp to ensure that products are safe for human consumption.

Regulating Cannabis and Hemp

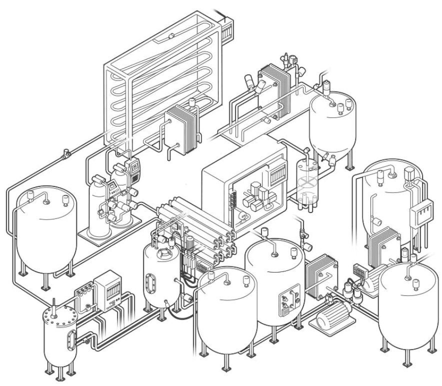


The lack of federal oversight with regard to heavy metals in medicinal cannabis products in the US has left individual states to regulate its use. Medical cannabis is legal in 34 states, while 11 states and Washington, DC allow its use for adult recreational consumption. However, the cannabis plant is known to be a hyper-accumulator of heavy metals in the soil, so it is critical to monitor levels of elemental contaminants to ensure cannabis products are safe to use.

Unfortunately, there are many inconsistencies with heavy metal limits in different states where cannabis is legal. The vast majority of states define four heavy metals: lead, arsenic, cadmium, and mercury. Some base their limits directly in the cannabis, while others are based on human consumption per day. Others take into consideration the body weight of the consumer, while some states do not even have heavy metal limits.

Certain states only require heavy metals in the cannabis plant/flower, while some give different limits for the delivery method such as oral, inhalation, or transdermal **(1).** This makes it extremely complicated, because currently all regulations apply only in the state where the cannabis is grown, processed, and sold. And since the federal government still considers cannabis a Schedule I drug (same as heroin), there can be no interstate commerce with regard to cannabis products. However in 2020, it will be legal to grow hemp (which contains less than 0.3 percent THC) anywhere in the US for the production of CBD-based and other industrial products, so it will be interesting to see how the Department of Agriculture regulates the industry at the federal level, when cannabis is regulated by the individual states.

What Can be Learned from the Pharmaceutical Industry?



So clearly there is a need for more consistency across state lines, particularly as the industry inevitably moves in the direction of federal regulation. The cannabis industry can learn a great deal from the pharmaceutical industry, as it went through this process over 20 years ago when it updated its 100-year old qualitative sulfide precipitation test for an undefined suite of heavy metals **(2)** to eventually arrive at a list of 24 elemental impurities in drug products using plasma spectrochemical techniques.

These procedures were described in the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines on elemental impurities **(3)**. These new directives defined maximum permitted daily exposure limits based on well-established elemental toxicological data for drug delivery methods (including oral, parenteral, and inhalation), together with the analytical methodology to carry out the analysis. This meant that pharmaceutical manufacturers were required to not only understand the many potential sources of heavy metals in raw materials and active ingredients, but also to know how the manufacturing process contributed to the elemental impurities in the final drug products.

The beginning of the journey to regulate elemental impurities in pharmaceuticals in the late 1990s can be likened to the production of cannabis and hemp derived products today, where the source of elemental contaminants is not fully understood. In particular, the elemental toxicological guidelines to regulate the cannabis industry are being taken very loosely from a combination of methods and limits derived by the pharmaceutical, dietary supplements, food, environmental and cosmetics industries. Even though the process of manufacturing cannabis products might be similar in some cases to drugs and herbal medicines, the consumers of cannabis and hemp products are using them very differently and in very different quantities, particularly compared to pharmaceuticals, which typically have a maximum daily dosage. The bottom line is that heavy metal toxicological data generated for pharmaceuticals over a number of decades cannot simply be transferred to cannabis, hemp, and their multitude of products.

An added complication is that the cannabis and hemp plant can not only absorb heavy metals from the soil, but also from contaminants in fertilizers, nutrients, pesticides and the growing medium as well as from other environmental pathways. Additionally, the process of cutting, grinding, and preparing the cannabis/hemp flowers for extraction can often pick up elemental contaminants from the stainless steel manufacturing equipment. Finally the cannabinoid extraction process will extract different amounts of heavy metals, depending on the solvent and/or the temperature and pressure used in the extraction/distillation process which could potentially end up in the finished products. In addition, some cultivators will use nutrients containing metal-based bud/flower enhancers, which would not be picked up by the state regulatory process. It's also worth pointing out that the equipment used to deliver these products to consumers such as inhalers, and vaporizers can mean the user is exposed to additional sources of elemental contaminants from inside these devices, apart from what's in the cannabinoid compound itself.

Phytoremediation Properties of Cannabis and Hemp

Cannabis and hemp are known to be hyper-accumulators of contaminants in the soil. That is why they have been used to clean up toxic waste sites where other kinds of remediation attempts have failed. In the aftermath of the Chernobyl nuclear melt down in the Ukraine in 1986, industrial hemp was planted to clean up the radioactive isotopes that had leaked into the soil and ground waters **(6)**. Of course Chernobyl is an extreme example of heavy metal and radionuclide contamination, but as a result of normal anthropogenic activities over the past few decades, including mining, smelting, and the spread of pesticides, heavy metal pollution has become one of the most serious environmental problems today. And with all the diverse and varied conditions used for growing cannabis, it will be very difficult to eliminate all these potential sources of pollution in order to reduce their impact on the plant's biology.

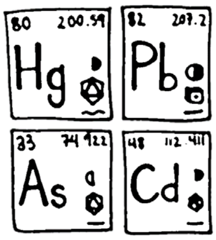
So there is no question that the current suite of four heavy metals being required by state-based regulators is totally inadequate to ensure cannabis products are fit for human consumption. Based on evidence in the public domain, there are about 15 heavy metals found in natural ecosystems and contamination from industrial activities that could be potential sources of contaminants in the plant, including nickel, vanadium, cobalt, copper, selenium, barium, silver, antimony, chromium, molybdenum, manganese, zinc, and iron. They might not all have a negative impact on the health of the plant during cultivation, but the chances that they will end up in the flowers and the final manufactured products are very high. Their levels of toxicity would need to be investigated further, but there is a case to be made that the majority of them could be the future basis of a federally regulated panel of elemental contaminants in cannabis and hemp **(7)**. For this reason, it is critically important to characterize all the potential sources of elemental contamination, including the cultivation of the cannabis/hemp plant, as well as the cannabinoid manufacturing process.

Testing Procedures

As heavy metals are likely to appear in some hemp and cannabis products, the correct sampling and testing procedures is absolutely critical, so the analytical result of the sample is actually indicative of the cannabis plant. The most suitable and widely used technique is considered to be inductively coupled plasma mass spectrometry (ICP-MS), which is a very sophisticated multielement analytical technique that can easily measure down to parts per trillion detection levels. However, it requires an analytical chemist with a high level of knowledge and expertise to fully understand the nuances of ultra-trace elemental analysis, including lab cleanliness, sources of contamination, sample preparation, digestion techniques, instrumental method development, interference corrections, calibration routines, use of reference materials, and validation procedures. In other words, in the hands of an inexperienced user, it could easily generate erroneous results. For that reason, the expertise of the testing lab and the people running the instrumentation is of prime importance.

The pharmaceutical industry went through this learning curve when it was first required to use plasma spectrochemistry after 100 years of the qualitative colorimetric sulfide precipitation test for heavy metals. As the leader of the heavy metals task force on the American Chemical Society's (ACS) reagent chemicals committee, I had become very familiar with the demands of the pharmaceutical community. My committee had worked very closely with the United States Pharmacopeia (USP) to align the ACS test for heavy metals using plasma spectrochemistry with the new USP methodologies, described in Chapters 232 and 233. It was very clear that pharmaceutical manufacturers were unfamiliar with working at the ultra-trace level required by techniques like ICP-MS. So this became the incentive to write, *Measuring Elemental Impurities in Pharmaceutical Materials: A Practical Guide*, which was published in the spring of 2018 **(8).** Once it was in the public domain, I then turned my attention to the cannabis industry and started talking to cultivators, growers, producers, processors regulators, and testing labs to get a better understanding of what the industry needed with regard to its heavy metals' testing requirements. As a result of that background research, in early 2019 I began the process of writing a new book, which focused on heavy metals in cannabis and hemp, which was published in September, 2020 **(9).** This article represents an overview of some of the initial findings of my research.

Potential Sources of Heavy Metals: Cultivation and Growing



Considerations about where to grow cannabis and hemp is going to be critically important because it could have serious implications on the level of heavy metals that are absorbed by the plant and its resulting safety for human consumption of cannabinoids. Historically, apart the Western and Southern regions of the US, much of the cannabis in the US has been grown indoors in greenhouses under controlled growing environments, so the absorption of heavy metals into the plant has been kept in check reasonably well.

But in 2020, it will be legal to grow hemp for CBD production anywhere in the US **(10).** As a result, it will become more challenging to keep the levels low, because most of the hemp plants will be grown outdoors on farms where the soil might be an additional source of contamination. So let's first take a look at all potential sources of elemental contaminants in cannabis and hemp, from a growing and cultivation perspective, and then we'll focus on the impact of the extraction, manufacturing and packaging processes.

Cannabis and Hemp: Avid Accumulators of Elemental Contaminants

Plant-based phytoremediation is emerging as a cost-effective technology to concentrate and remove elements, compounds and pollutants from the environment **(11).** And within this field, the use of cannabis and hemp plants to concentrate metals from the soil into the harvestable parts of roots and above-ground shoots (phytoextraction) has great potential as a viable alternative to traditional contaminated land remediation methods **(12).** However, the natural inclination of these plants to absorb heavy metals from the soil could potentially limits its commercial use for the production of medicinal cannabinoid-based compounds. A number of studies provide convincing evidence that cannabis is an active accumulator of heavy metals such as lead, cadmium, arsenic, mercury, magnesium, copper, chromium, nickel, manganese, and cobalt **(13, 14, 15)**

Other Pathways of Contamination

An added complication is that cannabis and hemp plants not only absorb heavy metals from the soil and growing medium, but also from contaminants found in fertilizers, nutrients, pesticides, and growth enhancers, as well as from other environmental pathways **(16, 17).** Additionally, the process of cutting, grinding and preparing the cannabis/hemp flowers for extraction can often pick up elemental contaminants from the manufacturing equipment.

It should also be emphasized that the extraction process has the potential to extract varying amounts of heavy metals, depending on the solvents and the super/sub critical extraction temperatures/pressures used. It's therefore critically important that an optimized extraction process is carried out in order to minimize the carry-over of heavy metals, even though it might not maximize cannabinoid yield **(18).** It's also worth pointing out that the equipment used to package and deliver these cannabinoid products to consumers, such as inhalers, vaporizers and transdermal patches can mean the user is exposed to additional sources of elemental contaminants, apart from what's in the cannabinoid compound itself **(19).**

Main Factors for Metal Uptake from the Growing Medium

The health and growth of all plants rely on the absorption of essential nutrients and minerals being available in the dissolved, ionic form in the soil. To maintain enough water to survive and thrive, the plant's primary means of facilitating the movement of water is through transpiration, which is a highly efficient means of drawing a concentrated solution of minerals and nutrients out of the soil. Transpiration works by the evaporative loss of water from the shoots, which is controlled by the opening and closing of specialized pores (known as stomata) embedded in the surface of the leaves that initiates gas exchange. When the stomata are open, the pressure potential of the plant becomes very negative, creating a vacuum effect that draws water and nutrients into the plant, moving it from the roots to the shoots. Unfortunately, this is also the mechanism that the plant draws in heavy metal contaminants in addition to the nutrients.

The rhizosphere is the region of soil in the vicinity of plant roots in which the chemistry and microbiology is influenced by their growth, respiration, and nutrient exchange **(20).** Unfortunately, under certain conditions the plant's root system will preferentially absorb heavy metals over other minerals, which cannot be explained exclusively by passive ion uptake. The hyper-accumulating properties of cannabis and hemp aren't fully understood, but are partially dependent upon other factors, including soil pH, availability of other metal/mineral ions in solution, the N/P/K (nitrogen/phosphorus/potassium) ratio and the ability of the plant's natural metalloproteins, which act as chelating compounds to bind with the heavy metals to reduce the rate of absorption and overcome their toxic effects.

It should also be noted that the plant's natural polyamine compounds will strengthen their defense response and enhance their protection against diverse environmental stressors including toxicity, and oxidative stress. Bode wrote an excellent article on the underlying mechanisms of heavy metal uptake by cannabis plants, which should be essential reading for the cannabis grower **(21).**

Based on evidence in the public domain, there are approximately 15-20 heavy metals found in natural ecosystems that could be potential sources of contaminants, including Lead, Arsenic, Mercury, Cadmium, Nickel, Vanadium, Cobalt, Copper, Selenium, Boron, Thallium, Barium, Antimony, Silver, Gold, Zinc, Tin, Manganese, Molybdenum, Tungsten, Iron, and Uranium. Many of these elements exist as different species (eg. metalloids), based on their oxidation state, organic/inorganic/ionic form **(22),** or more recently as engineered nanoparticles that could find their way into waste water streams **(23).** Their levels of toxicity would need to be investigated further, but there is a compelling case to be made that the many of these elements, metalloids, and speciated forms could be the future basis of a federally-regulated panel of metal-based contaminants in cannabis and hemp.

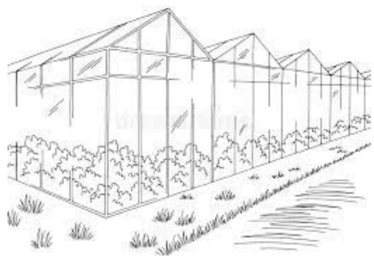
Outdoor Growing Sources

Let's take a closer look at some of the 'real-world' sources of elemental contaminants that are referenced in the open literature. It's only when cannabis and hemp cultivators and growers have a good understanding of these diverse range of issues, can they hope to reduce or even eliminate them. This isn't an exhaustive list, but it represents a good starting point to begin investigating the problem.

- When gown outdoors, if possible, the soil should be characterized to make sure that the elemental contaminants are at acceptable levels. Explore the use of natural chelating agent such as humic acid or synthetic ones like biochar to bind with the harmful metallic contaminants to minimize their uptake by the plant's root system **(24)**

- In areas where gold and silver mines are found, there is the potential for high mercury levels, as mercury amalgamation is a well-accepted extraction method **(25).** These mines are particularly prevalent in the Emerald Triangle growing states of California, Oregon and Washington, which have some of the highest density of outdoor cannabis farms in the US **(26).**
- All metal smelting plants will experience heavy metal contamination in surrounding areas. Lead and copper ores in particular contain high levels of arsenic **(27).**
- Environmental Protection Agency (EPA) superfund sites, especially those involved in the manufacture of weapons could have high levels of heavy metal/radioisotope contaminants in the soil **(28).**
- Fly ash waste from coal-fired power plants is extremely high in heavy metals from the coal combustion process. Surrounding areas where the fly ash has been dumped will likely be contaminated **(29).**
- Decades of using leaded-gasoline has contaminated much of the soil close to and around major highways and roads **(30)**
- Many industries are known to emit elemental mercury into the atmosphere, including coal fired power plants, metal refineries/smelters, petrochemical plants, and cement works. It's well documented by the Clean Air Act that approximately 100 tons of mercury are emitted by US industries annually, much of it being converted into the highly toxic methyl mercury **(31).**
- Wood preservation chemicals contain high levels of copper, arsenic, and chromium **(32).** Areas around these plants are likely contaminated.
- Low-grade fertilizers/nutrients made from phosphate rocks contain significant amounts of elemental impurities **(33).**
- Nickel has been promoted as a cannabis flower/bud-enhancer and silicon as a way of increasing shoot size, which means that higher levels of nickel and silicon will invariably end up in the cannabis product. These elements typically aren't required by the vast majority of states, so they would escape the scrutiny of most state regulators. **(34, 35).**
- Some inorganic pesticides contain arsenic, copper, lead, and mercury **(36).**
- Although there are no elemental species of arsenic and mercury defined in the current state-based limits for heavy metals, the pharmaceutical industry has shown that inorganic and organic forms of these elements should be monitored if the maximum limits for the total amount are exceeded. In addition, depending on where the cannabis/hemp plants are grown, will also dictate whether other speciated forms should be monitored. Examples of this might include the highly toxic hexavalent chromium (CrVI) compared to the relatively innocuous trivalent species (CrIII) **(37).**
- At some point in the future, nanoparticle characterization in the soil and uptake by the cannabis plant may be needed particularly when there are regulated methods for environmental and food-based assays **(23).**

Indoor Growing Sources



Although an indoor or greenhouse growing environment is far more controlled than cultivating plants outdoors, there are still many opportunities for picking up elemental contaminants. Nutrients, fertilizers and potable water supplies are three of the potential sources of metal contamination. As a result, indoors plants cultivated in a soil-based growing medium or hydroponically-grown, are highly dependent on the nutrients, minerals and water used. For that reason high quality fertilizers and a source of clean water are essential for healthy plants. Here are three areas of concern when growing plants indoors.

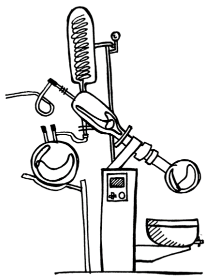
Last year the EPA estimated that 30 million people in the US live in areas where drinking water violated safety standards **(38).** The EPA defines a list of primary and secondary elemental maximum contaminants levels (MCLs) in drinking water and overseas all local water authorities/municipalities in the US to ensure compliance. It's important to know these levels in your region to make sure they are well below the limits for metal impurities (they will be posted online by your local water authority). Also keep in mind that these are levels for samples taken at the water treatment plant and not the levels measured at your home or growing site. EPA mandates that a municipality only has to measure water quality at its customers sites every two years and has to take remedial action only if 10 percent of those sampled are above the MCL. Look what happened with lead-contaminated drinking water in Flint, Michigan, because the source was changed from lake water (Lake Huron) to the local Flint River, without understanding the chemistry and its corrosion properties. As a result, the water dissolved the inside of the old lead pipes and ended up contaminated the drinking water supply **(39).**

- Decades of using lead, cadmium, and arsenic based pigments in residential and commercial paint has meant that many of the older homes and buildings still contain these types of paints, which have often been painted over, but still produce dust/particles that can potentially be problematic **(40).** If in doubt, scrape some paint off and get it tested.
- Some plasterboard used in the construction industry is made from gypsum-based flue gas desulfurization (FGD) waste products mixed with a silicate material known as clinker. FGD is produced by scrubbing particulate emissions from coal-fired power plants, which are notoriously high in heavy metals **(41).**

The pharmaceutical industry has shown that the only way to reduce elemental impurities is to fully understand all the potential sources. It took over 20 years to investigate every facet of drug production, including analyzing the raw materials, and active pharmaceutical ingredients, together with the entire manufacturing and packaging process. The US cannabis industry is currently nowhere near this level of knowledge. This is even more disturbing because our insatiable demand for cannabis products is being fulfilled by other parts of the world. This was exemplified in two recent newspaper editorials. One of them highlighted the gross soil contamination produced my metal refineries in many parts of China **(42).** That in itself isn't surprising, but the other editorial talked about Yunnan Province in southern China, which is now producing CBD-products for the US market **(43).** So the US cannabis and hemp industry must begin to find ways to reduce levels of heavy metal contaminants, otherwise consumers will turn away. If cannabinoid products on the market are considered unsafe to use, it might be too late by the time the FDA comes knocking at the door.

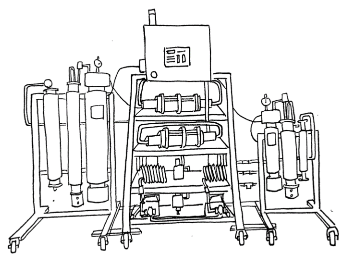
Let's now take a closer look at potential contaminants derived from and possibly enhanced by the production of commercially-available cannabinoid products.

Sources of Contamination: The Cannabinoid Manufacturing Process



With the approval of USP Chapter <232>, **(44)** Chapter <233>, **(45)** and ICH Q3D guidelines **(46),** the pharmaceutical industry was mandated to fully-understand elemental pathways of the entire drug manufacturing process, including impurities derived from the raw materials, excipients, active ingredients, organic synthesis method, water quality, manufacturing equipment, mixing vessels, containers, and packaging etc. To comply with these directives, companies had to show convincing evidence (data and/or risk assessment studies) to the regulatory agency that up to 24 elemental impurities of toxicological concern are below certain maximum permitted daily exposure (PDE) limits for the major routes of delivery (oral, parenteral, inhalation, transdermal). The process was challenging, painful and sometimes confusing, but after a 20-year approval process, they eventually met the challenge by generating the necessary data to show that drugs were safe to use.

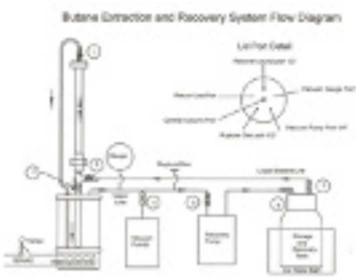
Similarities Between Production of Pharmaceuticals and Cannabinoids



Unfortunately, the cannabis and hemp industry is moving so rapidly that few companies are taking the time to investigate potential sources of heavy metals throughout the entire manufacturing processes of the multitude of cannabinoid products on the market today. It is well-accepted that cannabis and hemp will absorb heavy metals from the growing medium, soil, nutrients, and fertilizers. However, at present, there is less understanding of what heavy metals contaminants are carried over into the pure cannabinoid extracts from the various purification steps, including preparation, extraction, evaporation, concentration and distillation. It makes sense that there is some degree of transfer from the plant to the extract, but how do the extraction processes and the solvent properties have an impact on the amount carried over?

To better understand all these potential sources of contamination in the production of cannabinoid products, the cannabis plant can be considered very similar to the raw materials used in pharmaceutical manufacturing; while the various cannabinoids can be likened to the active pharmaceutical ingredients (API) used in the drug formulation. The similarities are quite striking, because pharmaceutical manufacturers were required to characterize the entire production process to reduce elemental impurities in drug products. For that reason the cannabis industry clearly has to better understand the entire cannabinoid production process including the cultivation and extraction steps in order to reduce heavy metal contaminants in the final products.

Cannabis Extraction Process



Extraction is necessary to purify and concentrate the essential cannabinoid compounds from the plant while also removing the undesired contaminants. These compounds are mainly contained in the female flower's trichomes, small glandular hairs protruding from the surface of the plant, which secrete a sticky resin from the cells at the end of the trichome, containing most of the cannabinoids and terpenoids of interest. When the optimum extraction method is employed it can either result in pure, isolated compounds or more natural, full-spectrum extracts containing a wide array of the cannabinoids and terpenoids found in the source material. Most consumers of cannabis are familiar with delta-9- tetrahydrocannabinol (THC) and cannabidiol (CBD) but these are only two of the 100+ cannabinoid compounds found in cannabis. The ability to extract the desired compounds allows medicinal products to be manufactured based on the desired therapeutic effect for the specific ailment being treated. However, cannabis also contains 140+ different terpenes (terpenoids), aromatic compounds best-known for giving cannabis its distinctive fragrances and flavors. Terpenes are currently gaining a great deal of attention not only for their potential therapeutic value, but also because of the so called "entourage effect" when combined with other cannabinoids.

The technology needed to extract bioactive compounds from the flower's trichomes, or other parts of the plant, clearly depends on medicinal product goals. It's also important to emphasize that when cannabis is harvested, it contains practically no THC and CBD. There are, however, significant amounts of tetrahydrocannabinolic acid (THCA), and cannabidiolic acid (CBDA). So to convert THCA and CBDA to THC and CBD, the cannabis must first be heated to remove the carboxyl functional group (COOH) from the respective THCA and CBDA molecules. This process is known as decarboxylation, which converts them into THC and CBD respectively. These chemical structures are the gateway molecules to the human endocannabinoid system (ECS) that runs throughout the central nervous system, delivering the desired therapeutic/psychoactive effect to the consumer.

It's therefore clear that heat is a very efficient way to increase the bioavailability of certain compounds in cannabis; however, it does not give us the ability to select which compounds we want to activate. This is achieved by carrying out extraction procedures using different organic solvents, often combined with precise control of temperature and pressure, which allows for the optimization and fine-tuning of the products being made.

Different Extraction Approaches

The methods used to extract cannabinoids are as varied as the compounds themselves. Some techniques use temperature and pressure, relying on thermal and mechanical forces to remove valuable compounds from the plant's trichomes; others rely on organic solvents to carry the desired compounds into another solution, which is then processed again to remove the solvent. Some even use microwave- and ultrasonic-assisted extraction methods **(47).** Whatever extraction technique is employed, they all use a combination of solvent, temperature, pressure, and time, in a precise, controlled manner, to access one or many of the cannabinoids, flavonoids, and terpenes present in the cannabis plant. There are a myriad of different extraction techniques, all with their own strength and weaknesses. Let's take a closer look at three of the most common approaches **(48).**

Alcohol Extraction

Alcohol extraction is one of the most efficient extraction methods for processing large batches of cannabis flower, and can be done in hot, cold, or room temperature conditions. Typically carried out using hot ethanol (or propanol), extraction is generally accomplished using the Soxhlet extraction technique, which cycles the hot solvent through the solid cannabis flower, stripping the cannabinoids and terpenes from the flower in the process. However, the method can be difficult to scale up to large batches, and often extracts unwanted chlorophyll and plant waxes from the cannabis flower due to the polarity of the ethanol solvent that often requires several additional post-processing steps (filtering, distillation, evaporation etc.). Cold ethanol or even room temperature helps to avoid this problem, as the cooler temperatures make it a little more difficult for the unwanted polar plant waxes and chlorophylls to dissolve in a polar ethanol solvent.

Hydrocarbon Extraction

Hydrocarbon extraction, normally achieved using butane or propane, is able to extract a greater variety of terpenes from the cannabis material than the ethanol extraction method. For products such as vape oils or oral tinctures, where the cannabis extract is unlikely to be masked by other flavors, preserving these terpenes helps to give the extract a specific flavor and aroma.

This improved extraction comes as a result of the low boiling point of the hydrocarbon, (butane, -0.5°C) at standard pressure. After cold butane solvent has washed over the cannabis plant material and extracted its oils, the solvent can be easily cold-boiled off to leave oil which is more representative of the entire plant as more of the temperature-sensitive terpenes will be retained.

However, like the ethanol method, hydrocarbon extraction cannot be so easily be scaled up to deal with large single batches of cannabis material. While the low boiling point of butane is advantageous when the solvent needs to be removed without removing any other organic compounds, these flammable solvents also present a safety hazard to workers. Hydrocarbon extraction is a very hands-on process and is rarely automated, meaning that there is almost always an operator in close proximity to the extraction vessel. In the interest of safety, hydrocarbon extraction is done on a much smaller scale, though the speed and efficiency of this extraction method means its overall output still makes it suitable for large-scale operations.

Super/Sub Critical CO_2 Fluid Extraction

Super- or sub-critical CO_2 fluid extraction is relatively new to the cannabis industry, but it's already becoming a popular choice. In brief, the method involves using specialized pressure and temperature control equipment to turn gaseous CO_2 into a super or sub-critical fluid. When passed over cannabis material, the fluid can easily extract waxes and oils from the cannabis plant. Super-critical fluid extraction refers to a higher temperature and pressure, which is good for THC/CBD yield, but tends to extract more of the non-targeted compounds including contaminants. While the subcritical method uses a much lower temperature and pressure, which sacrifices yields, but leaves many of the contaminants behind

When cannabis is processed under relatively low pressures and temperature conditions over a longer period of time, the amount of post-processing that's required after extraction is minimized, and can usually be used without any further processing. When using higher temperature and pressure conditions, winterization is often used to clean up the extract and remove unwanted waxes and fatty acids. This is achieved by soaking the extract in cold ethanol (-20°C) for approximately 24 hours and then filtering out the unwanted solid waxes and lipids.

The major downside of CO_2 extraction is the high initial equipment cost which can be prohibitive for start-ups or small businesses. However, unlike ethanol or butane, CO_2 is a very flexible and tunable solvent, which can pull unique compounds from botanicals using different pressures and temperatures. In addition, CO_2 is far safer than the flammable hydrocarbon methods. It is also worth noting that more often than not, butane extraction results in a more concentrated product, which can be detrimental if the cannabis material contains toxins or contaminants from the cultivation process.

Rigorous testing

As mentioned previously, cannabis plants are avid accumulators of heavy metal contaminants in the growing medium, soil, fertilizer and other environmental pathways, which can end up being concentrated in the final extract. This can be significantly compounded if a further preparation, distillation, evaporation, or additional concentration step are required, using metal processing equipment and storage containers, which are potentially additional sources of contamination. For this reason, extraction and processing techniques must be supported by rigorous scientific testing from the very start of the process production cycle, which means that the best practices in cannabis extraction often start at the growing stage.

Cannabis that is grown in controlled environments and under strict quality control usually produces the purest extracts. In other words, if the cultivation of the plant is carried out indoors using clean, uncontaminated growing medium, high purity nutrients and fertilizers and ultra-clean water, it's probably going to result in lower levels of elemental contaminants in the plant. Of course, this is not always going to happen, but will likely be the case compared to plants cultivated outdoors, which could pick up significant levels of heavy metals if grown in, near or around contaminated soil or if low quality fertilizers are used.

Extraction objectives

The optimum extraction method is often selected based on what cannabinoid/terpenoid combination is required, which is typically chosen based on the required medicinal product or desired therapeutic/psychoactive outcome. In other words, a processor doesn't decide on whether they are going to use CO_2, butane, ethanol, or another extraction process. Instead it's driven by what isolate/concentrate they are trying to make, based on the desired finished product. Whether it's a vape pen, a gummy, a cookie, a tincture or an oil, it begins with the final product and then it's "reverse engineered" to get the ingredients for those products and then finally selection of the extraction method that will best provide those ingredients.

This fundamental "reverse engineering" principle can even be related back to the cultivar as it's important to select the plant that will provide the desired molecular profile or to manipulate the chemistry to get the desired ingredients. David Hodes wrote an excellent review of the major commercial extraction methods and the pros and cons of using each approach, which is highly recommended reading for any current or new processor who wants to optimize their extraction procedures **(49).**

Heavy Metal Absorption Mechanisms

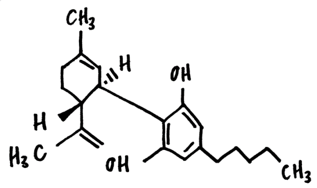
As discussed earlier, the cannabis plant takes up heavy metals through its roots from the growing medium, where it's eventually stored in the shoots, leaves, and flowers. Khan and coworkers wrote a very informative paper on tracing the movement of heavy metals from the soil into the cannabis plant and investigating which part of the plant they eventually ended up **(50).** Let's just recap how this mechanism takes place.

The transport of minerals from the growing medium into the cannabis root is an active process that is energy dependent. We know that the concentration of essential minerals in root tissue is significantly higher than the surrounding soil, making this concentration difference unfavorable for passive transport. By pumping protons out of the root cells, the positive charge of the soil increases creating a gradient that drives desirable cations into the plant cells. Unfortunately, these transport channels don't exclude chemically similar ions making them a possible entry point for heavy metals in the soil.

Once they have entered the plant tissue, these minerals and/or heavy metals are secreted into the xylem by nearby cells. The xylem is mainly used for transporting water from roots to stems and leaves but will also transport other metal ions in solution. Once these metal ions have entered the xylem, they are swept up by the transpiration process and distributed throughout the plant, in many cases, bound up with chlorophyll molecules. Some of the ions will move laterally throughout the roots and stems, while others will continue on to the leaves where they are released into the atmosphere through the stomata. However, most of the metal ions will be actively transferred to the phloem, which is responsible for transporting food produced from photosynthesis to other parts of the plant, including the reproductive organs, the flowers, and the trichome cells **(51).**

Traditional Pathways

Unfortunately, many of the heavy metals found in the soil, exist in different oxidation states, metalloid forms, organo-metallic species, and possibly nanoparticles. Some are impacted by nitrogen, phosphorus and potassium (NPK) from the nutrients/fertilizers, while others are



dependent on the soil ph. And some even end up being complexed with natural chelating agents in the soil, like humic acid or bound up with other transition metals such as iron and manganese.

Additionally, the plant's own polyamine compounds will strengthen its natural defense mechanism against diverse environmental stressors such as metal toxicity and oxidative stress. The journey of elemental contaminants from the growing medium to the cannabis flower isn't completely understood – it's an area of research that has not been fully explored. We can only go on what is known about traditional agricultural plants and botanicals, and make the assumption that they are very similar, which means that the elemental movement via the cells and other biochemical pathways are varied and diverse based on the growing conditions and the individual metal species. However, it can be fair to assume that the trichomes of the female cannabis flower, which are home to 100+ cannabinoid compounds, also contain many heavy metal contaminants derived from the growing process.

What's More Important: Low Heavy Metals or High Potency Yield?

It's well-recognized that many metal ions and species are only partially soluble in organic solvents, but this is going to be dependent on a combination of the specific metal ion, species and oxidation state, the polarity and boiling point of the solvent, and the extraction temperature and pressure used. This begs the question, what is the optimum extraction technique to minimize the heavy metals carried over but to maximize the cannabinoid yield? It's well-accepted that most cannabinoids are not very water soluble, so what is the right balance with regard to solvent choice and polarity to optimize this extraction process. Unfortunately, there is very little information in the public domain on this topic. I thought there might be a comparison between heavy metal levels in cannabis flowers and the resulting extracted concentrate, but unfortunately my research was not fruitful. For that reason, I suspect there has been no such study carried out and processors have not fully investigated the problem. It appears that everything is geared towards maximum potency yield and they just hope that most of the heavy metals are left behind in the extraction/distillation process and are not being co-extracted/co-distilled with the cannabinoid.

This is probably a sound strategy if the plants have been cultivated indoors, where the growing conditions are far more controlled and the heavy metals in the plant should be relatively low. However, that is not always the case. For example, some indoor growers are now using organic fish emulsion/hydrolysates, which are notorious for containing high levels of mercury **(52).** This is predominantly a result of bioaccumulation up the food chain from the smaller bottom feeders to the large predatory fish. The mercury is typically environmental fallout from industrial activity (power plants, metal refineries etc.), which ends up in the sediment of ponds, rivers and lakes and often gets converted to methyl mercury (CH_3Hg), which is even more toxic than the elemental form **(53, 54).**

We are also now beginning to see CBD products derived from hemp in the marketplace, which is predominantly grown outdoors, where the cultivation conditions are less controlled. This leads to the conclusion that heavy metals in plants grown outdoors are potentially going to be much higher. For these reasons, there clearly needs to be a scientifically driven investigation to better-understand the level of heavy metal movement from the plant through each step of the preparation/extraction/distillation/concentration process? I'm very hopeful that a concerned processor, university, or research organization will take up this challenge.

Manufactured Product Recalls

There is clear evidence in the public domain that heavy metals are not being completely removed from commercially-available cannabinoid products, evidenced by the fact that from time to time we see products recalled for high levels of heavy metals. The following stories, taken from online newspapers and news sites over the past 6-12 months are examples of this.

- There was a case recently where a CBD oil producer from Florida was forced to recall a CBD tincture by the FDA because it had 10x higher Pb levels - 4.7 ppm compared to the maximum allowable limit for Florida which was 0.5 ppm **(55).**

- And there have been other similar stories reported where a CBD manufacturer claimed their product was "heavy metal" free but on further testing, it was found to be over the legal limit for Pb, Cu and Ni. This case is currently going through the litigation process. **(56).**
- Thirteen strains of medical marijuana sold at a licensed medical marijuana center in Michigan were recalled. They failed to meet state maximum allowable limits for heavy metals when tested, because they were contaminated with high levels of Cd and As, according to the Michigan Bureau of Marijuana Regulation MBMR) **(57).**
- Ohio state regulators recently recalled medical marijuana flower because it was packaged for sale at dispensaries before being tested for high levels of heavy metals, in addition to microbes, mycotoxin fungi and foreign matter **(58).**
- Following an inspection by the California Bureau of Cannabis Control (CBCC) a cannabis testing lab had to give up its business license. The inspection uncovered false results for a group of contaminants during cannabis product testing over a four month period. This was especially detrimental to the California cannabis industry because they were in the process of putting in stricter limits for toxins, such as heavy metals. **(59)**
- Medical cannabis regulators in Maryland expanded testing for heavy metals in marijuana products as they warned the public about the risk for possible lead contamination in popular vaping devices. They issued an advisory to notify patients and other stakeholders of potential lead contamination of cannabis liquids in vape cartridges following exposure to heat inside the device. **(60)**
- Lead and ethanol contaminants were found in vape cartridges sold in Hawaii. They don't say where these contaminants come from, but the lead most likely comes from the soldered battery connections inside the vaping device, while the ethanol is a carryover from the cannabinoid extraction process. At vaping temperatures of approx 200°C, who knows what these contaminants are doing to someone's lungs, so it's not surprising that a local doctor thinks they might be linked to a recent spate of lung illnesses **(61).**

These are many more stories like this in the public domain, emphasizing that there is a continuing trend of products that are testing above maximum allowable levels for heavy metals, which indicates that growers and processors do not fully understand how heavy metals end up in commercially available cannabinoid products. It should also be pointed out that the majority of states that allow for medicinal cannabis only regulate Pb, As, Cd and Hg. However, there is convincing evidence in the public domain that there are probably another 10-15 heavy metals that should potentially be considered when assessing contamination in cannabis and cannabinoid products. Currently, if these metals found their way into cannabis products, they would escape the scrutiny of most state regulators.

CBD-Based Drugs

There are many over the counter CBD-based drugs/ medications on the market for medicinal purposes, but there is only one CBD prescription drug that is regulated by the FDA. Epidiolex™ is a prescription CBD-based drug for seizures in young children, which is manufactured by G.W. Pharmaceuticals. And, because it a prescription drug, it is regulated according to USP Chapter 232/233 in the US and to ICH Q3D guidelines in the ROW for 24 elemental impurities Permitted Daily Exposure (PDE) limits. However, as the elemental impurities are not listed on the Epidiolex label, the only evidence I could find that indicates the level of heavy metals in the product is with regard to the company's patent application for their cannabis extraction process **(62).** A summary of its production/extraction protocol is shown below, which uses CO_2 with sub-critical fluid extraction conditions at a temperature of 10° C. and a pressure of 60 bar. On further investigation of the patent, the final heavy metal content is in the order of 20 ppm, although they do not mention what individual heavy metals this represents.

- Botanical raw material (dried cannabis) is decarboxylated by heating to approximately 105°C for 15 minutes, followed by approximately 145°C for a minimum of 55 minutes for THCA and 90 minutes for CBDA (Note: refer to the earlier section on decarboxylation).
- Extraction with liquid food grade carbon dioxide (CO_2) for up to 10 hours.
- Sub critical extraction conditions: approximately 60 bar pressure at 10°C
- Removal of CO_2 by depressurization to recover crude extract
- Winterization of crude extract by dissolution in ethanol followed by chilling solution (-20°C up to 52 hours) to precipitate unwanted lipids

and waxes

- Removal of unwanted waxy material by cold filtration (20 µm filter)
- Removal of ethanol and water from the filtrate by thin film evaporation under reduced pressure (60°C with vapor at 40°C - 172 mbar and 72 mbar)
- Final botanical drug substance (CBD extract) stored at -20°C.

By reading through the entire patent, the following text gives their reasoning for using this extraction procedure.

"….*the density of sub-critical CO_2 is low, and remains low even as pressure is increased until the critical point of the system is reached. Thus, whilst the solvating power of sub-critical CO_2 is reduced, a high degree of selectivity can be achieved, as only the most soluble components are efficiently dissolved by the CO_2; in this case the cannabinoid fraction. The result is the production of a relatively simple extract containing, as well as the cannabinoids, only a limited number of non-target compounds (inc. heavy metals), many of which can be removed relatively easily by a simple winterization clean-up step. In contrast, at higher temperatures there is a significant increase in the density of the CO_2 as it now exists in a supercritical fluid state. This has the effect of greatly increasing the solvating power of the solvent, which whilst generally advantageous in that more cannabinoids are solubilized, thereby giving high yields, in fact proves disadvantageous because the decreased selectivity of the more powerful solvent results in increased solubility of a range of non-target compounds which makes the resulting extract more difficult to purify.*"

Using this process, the resulting product specifications are shown in Figure 1:

As mentioned previously, the manufacturer

Specification for the control of BDS high in CBD:

Test	Test Method	Limits
Appearance	In-House	Brown viscous semi-solid
Identification:		
A	TLC	Spots have characteristic R_f and colours, compared with CBD standard
B	HPLC/UV	Positive for CBD
CBD content	In-house (HPLC-UV)	NLT 55% w/w of extract
Related cannabinoids:	In-house (HPLC/UV)	
THC content		NMT 7.5% of the CBD content
Others (total)		NMT 5% of the CBD content
Aflatoxin:	TBA	NMT 4 ppb
Total Heavy Metals:	Ph.Eur.	NMT 20 ppm
Residual solvents:	In-house	
Ethanol		NMT 5% w/w
Microbial:	Ph.Eur.	
TVC		NMT 10^5 cfu/g
Fungi		NMT 10^4 cfu/g
Other enterobacteria & certain other gram negative organisms		NMT 10^3 cfu/g
E. coli		Absent in 1 g
Salmonella		Absent in 10 g
S. aureus		Absent in 1 g

Figure 1: Product Specifications for a CBD extract manufactured by GW Pharmaceuticals (62).

claims that by using this extraction method, the total amount of heavy metals in the extract is kept low (< 20 ppm total). However, it's important to emphasize that they grow the plants indoors using pesticide-free compost in a controlled environment using potable, high quality water and fertilizers, without the use of any synthetic pesticides or herbicides. So even under these strict indoor growing conditions, where do these heavy metals come from? Imagine how difficult it would be to control all these variables with plants gown outdoors!

This is the only data I could find which shows heavy metal levels in a CBD extract. But we also must keep in mind that Epidiolex is a prescription drug which is regulated by the FDA. This means that to comply with USP directives and ICH guidelines, they have to show that 24 elemental impurities are below maximum PDE limits) for an orally-delivered drug compound. So clearly they have a strong incentive to ensure elemental contaminant levels are kept at a low level, as this prescription is being used on young, sick children with compromised immune systems.....not sure the same strict guidelines are used for cannabinoid-related products that are regulated by US states.

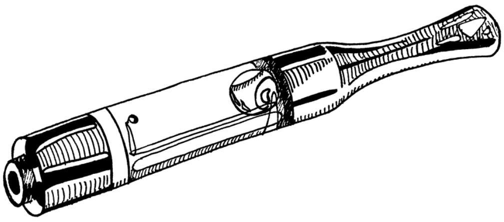
Lessons Learned

So it's worth going through a similar exercise as we did with the cultivation process and list a set of mitigation techniques in order to reduce potential sources of heavy metal contaminants from a cannabis preparation, drying, extraction, production and manufacturing perspective.

- Try to minimize the use of metal-based processing/extraction /manufacturing equipment. Perhaps there is a plastic alternative? In particular, avoid the use of stainless steel mixing vats, processing vessels, cutting blades and grinding equipment. Depending on the quality/specification of the stainless steel, metals ions and/or fine particulates of iron, chromium, nickel, cobalt, manganese, molybdenum and silicon could find their way into the processed cannabis flowers and eventually into the extracted oils and concentrates **(63).**
- If possible, use an optimized solvent and extraction/distillation process to minimize the amount of heavy metals ending up in the extracted products **(64).** It would also be useful to characterize the heavy metal content at every step of the manufacturing process from the cannabis plant through to the final product.
- Use ultra-clean solvents, and chemicals (low in heavy metals) for the extraction, distillation, concentration and infusion of cannabinoids from the plants **(65).**
- Make sure the source of water used in the cannabis production process is contamination-free. Minerals or elemental impurities in the water supply should be below the EPA maximum contaminant levels (MCL), otherwise the extracted material could pick up heavy metals from the water. A contaminated water supply could be real concern with older buildings that potentially have lead pipes, or copper/iron pipes connected with lead-based solder **(66).**
- Some heavy metals are leached out from delivery devices, such as vaping sticks, inhalation devices and infused transdermal patches. In particular, ensure that internal parts of vaping devices such as battery connections, atomizer, tank, and mouthpiece are not made from metallic components that could corrode at elevated vaping temperatures and deliver elemental contaminants such as Pb, Cr, Ni, Co and Fe to the consumer **(67).**
- Some silicate glasses and inexpensive plastics are notorious for elemental contamination, which could leach out into the product during storage. Even some cannabis rolling papers contain elemental impurities **(68).**
- Raw materials used in cannabinoid tablets, gel caps, creams, oils, edibles, and drinks formulations, that could possibly be contaminated with heavy metals. Example of this would be fillers, excipients, dissolution raw materials which are added to the tablet formulations, or diluent/mineral oils used in vaping liquids. Historically elemental impurities have been found in some dietary supplements **(69).**
- Any products or ingredients that come from Asia can potentially be a source of contamination. Think melamine in infant formula/pet food, lead-paint on toys, and lead in fake silver jewelry. In particular, it has been shown that inexpensive vaping cartridges, many which are sourced in China, contain metallic components that can corrode when vaped **(70).**

The Smoking/Inhaling of Cannabis

It's worth pointing out that historically, most consumers of recreational cannabis have used it by the inhalation/smoking route. Smoke chemistry has been predominantly investigated in tobacco products but many studies over the past ten



years have highlighted the qualitatively similar carcinogenic chemicals contained within both tobacco and cannabis smoke **(71).** In a recent study the International Organization for Standardization and Health Canada analyzed tobacco and cannabis cigarettes. The heavy metals contained in both smoked products included: mercury, cadmium, lead, chromium, nickel, arsenic, manganese and selenium **(72).** Quantitatively, there were lower heavy metal concentrations in cannabis smoke condensates, due mainly to the fact that the cannabis supply was grown hydroponically. In addition, the soilless growth medium of the cannabis plants required water and water-soluble hydroponic vegetable fertilizers which contain nitrogen in the form of nitrates. So, with no soil-based heavy metals to be extracted during the growth cycle of the cannabis, it was the liquid fertilizers used in the hydroponic systems that contributed mostly to the heavy metal levels. There is a great deal of information in the public domain about heavy metals in tobacco and tobacco products, such as nicotine electronic nicotine delivery (END) systems **(73).**

However, the more common way of inhaling cannabis products today is via vaping sticks, carts or pens. These devices are being marketed to the younger consumer by giving them fruit and peppermint flavors. The demand for these vaping devices is almost out of control and as a result, very loosely regulated, which is attracting manufacturers with very little regard for safety. There are literally thousands of these devices in the marketplace where the cannabis extract or oil is heated up to about 15-200 °C and the aerosol is vaporized into the consumer's mouth, very much like an inhaler for an asthma sufferer. The problem with this mode of delivery is that many of the components inside these vaping devices are typically metal, including the liquid tank, coil, mouth piece and battery terminals, which are usually made from materials such as stainless steel (Fe, Cr, Ni, Co), brass (Cu, Zn), nichrome (Ni, Cr) and soldered battery connectors (Pb, Sb, Sn). This means that at these kinds of temperatures, dissolved metals or even fine metallic particles will almost certainly be delivered to the consumer's air pathways and lungs via the mouth **(74).**

This is of great concern, because unlike oral delivery through the mouth and gastro intestinal digestion system, the lungs and respiratory system were designed to allow us to breathe. They bring oxygen from the air into our bodies and send carbon dioxide out. Air enters the respiratory system through the nose or the mouth. If it goes in through the nostrils there are tiny hairs called cilia that protect the nasal passageways and other parts of the respiratory tract, filtering out dust and other particles that enter the nose. There is no such filtering system in the mouth, so if any metal particulates are inhaled, there is no mechanism to stop them entering through the respiratory system into the lungs, where they can do serious damage, particularly if vaping is carried out on a regular basis over extended periods of time.

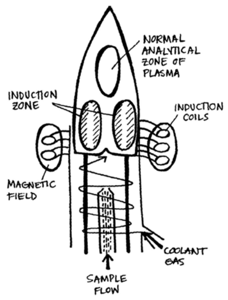
The recent nationwide outbreak of lung injuries and deaths by consumers using e-cigarettes, and/or cannabis vaping product that contain vitamin E acetate is a tragic testament to this fact. It's also worth pointing out that most state-based regulations only specify Pb, As, Cd and Hg, and as a result, would not identify the other elements (Fe, Cu, Zn, Cr, Ni, Sb, and Sn) if they were present in the vaping liquid because there is no requirement to test for them.

Understanding the Role of the Extraction Process

It took the pharmaceutical industry almost 25 years to fully-understand all the sources of elemental impurities in the manufacture of drug products. The cannabis industry has a great deal to learn about this process. The way to minimize heavy metals in cannabis and cannabis products is to first understand and characterize the cultivation process. Unfortunately, this is often very challenging, particularly if the plants are being grown outdoors. However, by carefully selecting the right cultivars and optimizing the extraction method, processors have the ability to reduce levels of heavy metals in the final cannabinoid extracts. Very often there are many choices when selecting an extraction technology, depending on the desired extract or the products being made for a therapeutic outcome. It is clear that elemental contaminants can be minimized by optimizing the entire production process including extraction solvent, temperature/pressure conditions and the other techniques used including evaporation/

distillation/filtration. This can also be extended to include the packaging and delivery systems, which are all important to characterize heavy metals in order to ensure that all cannabis products are safe for consumption.

Let's now focus on the heavy metal measurement process and how it could potentially lead to errors if not approached with extreme care.



Contamination and Errors Associated with the ICP-MS Testing Procedure

The potential for elevated levels of heavy metals being present in some hemp and cannabis products has meant that testing for elemental contaminants is absolutely critical. The most suitable and widely used technique is considered to be Inductively Coupled Plasma Mass Spectrometry (ICP-MS), which is a very sophisticated multielement analytical technique that can easily measure down to ppt (parts per trillion) detection levels. However, it does not preclude the use of other techniques such as ICP-OES (Optical Emission Spectrometry), Flame atomic absorption (FAA), electrothermal atomization atomic absorption (ETAA) or even atomic fluorescence (AF) as long as the detection capability is low enough for the required maximum limits defined by the state. It is also worth emphasizing that ICP-MS, with its superior sensitivity over other atomic spectroscopic approaches, is probably the best suited, because it is a multielement technique and even though today there are just four heavy metals required by most states there is a strong possibility that when the FDA has oversight of the cannabis industry, that list will be expanded to at least 10 additional elemental contaminants, and maybe more if the pharmaceutical industry is any indicator.

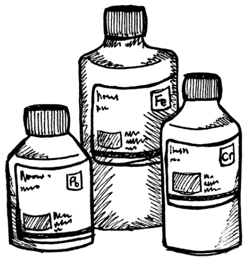
Benefits of ICP-MS for Testing Cannabis Products

There are approximately, 20,000 ICP-MS systems being used around the world for a wide and diverse range of trace element applications. However, even though it is very powerful technique with unparalleled sensitivity, it requires a skilled analytical chemist with a reasonably high level of knowledge and expertise to develop and run methods and to fully-understand the nuances of ultra-trace elemental analysis. Of particular importance in the real-world applicability of ICP-MS is a full understanding of lab cleanliness, sources of contamination, sample preparation, digestion techniques, instrumental method development, interference corrections, calibration routines, use of reference materials and validation procedures. In other words, in the hands of a novice or inexperienced user it could easily generate erroneous result leading to both false positives and false negatives **(75)**.

For that reason, the expertise of the testing lab and the personnel running the instrumentation is of prime consideration, and in particular they need to have an intimate knowledge of working in the ultra-trace environment, and to be aware of all the potential sources of elemental contaminants and to use robust validation procedures to ensure high integrity of the data **(76)**. This is exactly what the pharmaceutical industry was faced with in the US when they abandoned the old USP Chapter 231 sulfide precipitation colorimetric test **(76)** and wrote two brand new methods – USP Chapter 232 **(77)** to define 24 elemental impurities of toxicological concern and USP Chapter 233 **(78)** to address the most suitable analytical procedure including plasma spectroscopic technique, sample digestion procedure and robust validation protocols using standardized spike recovery testing procedures. The backbone of this new USP Chapter 233 was USP Chapter 730 **(79)**, which describes the use of plasma spectrochemistry and USP Chapter 1225 for the Validation of Compendial Procedures **(80)** for pharmaceutical-related samples. Note: A risk assessment strategy was also allowed by the USP and other global pharmacopeias, described in ICH Q3D guidelines **(81)** as long as compelling evidence could be demonstrated that an elemental impurity should be excluded from the list.

Laboratory Testing Procedures

Before we venture into what is required from a cannabis testing lab to be proficient in using highly sophisticated analytical equipment like ICP-MS for measuring ultra-trace levels of heavy metals in cannabis, let us take a closer look at the inherent limitations in the way it is carried out today. Consumers are asked to trust product ingredients, dosing suggestions and claims based on what the



producers tell them. An informed consumer might ask for a Certificate of Analysis (CoA) from the producer to show third-party lab test results.

However, the problem with this is that there is very little required standardization across different testing facilities. This was exemplified in a recent case I mentioned earlier where a CBD manufacturer claimed their product was "heavy metal" free but a concerned consumer got it tested and found to be over the state's legal limit for lead, copper, and nickel. This case, which is currently going through litigation highlights the problems that cannabis testing labs are not being seriously regulated, meaning there is no set of standardized protocols for equipment, operating procedures, certifications or qualifications of lab personnel. So one lab might get results that are below the state-based maximum allowable limits, while another lab shows higher results - which one is correct?

Accreditation Programs

Cannabis testing labs currently do not come under the umbrella of the FDA, so they have to be guided by state regulators who often do not have the necessary inspection expertise. Without standardization across all facilities, test results can be wildly inconsistent. What is the value of a CoA from a third-party lab that does not meet any kind of standards themselves?

However, as they begin to gain more experience, state regulators are attempting to put in place basic accreditation measures for cannabis labs to be licensed. One such approach is to implement the International Organization for Standardization (ISO) laboratory competence certification accreditation system, which in conjunction with the International Electrotechnical Commission (IEC), is responsible for ISO/IEC 17025:2017, a standard for calibration and testing laboratories across the globe, ensuring technical competence and the ability to produce precise and accurate test and calibration data. In addition, ISO/IEC 17043:2010, specifies general requirements for the competence of providers to develop and operate proficiency testing schemes using well-established interlaboratory comparison studies.

But the testing of cannabis and cannabis products requires specialized sample preparation and methods of analysis that can be extremely challenging for new labs and novice users due to its complex biological and biomolecular composition. ISO/IEC accreditation standards can provide confidence to cannabis consumers that testing is being performed correctly and to a universally accepted standard. To qualify for the accreditation, laboratories must conform to the ISO standards in all areas, including analytical procedures, calibration of instruments and equipment, as well as properly staffed and trained technicians who have met specific academic credentials. Overall, these required qualifications can be costly and time consuming, which may deter many of the start-up cannabis labs to invest in the necessary infrastructure to ensure they meet these high standards. So, to satisfy the demands of the industry, specialized organization like Emerald Scientific and others are developing Inter-Laboratory Comparison and Proficiency Test (ILC/PT) programs specifically for cannabis and hemp testing facilities, establishing much needed standardized protocols for testing **(82).**

More recently, the National institute of Standards and Technology (NIST) is spearheading a multi-phase project to encourage best practices in lab testing. The program called CannaQAP (Cannabis Quality Assurance Program) is meant to help laboratories accurately measure key chemical compounds in cannabis and cannabis products including oils, edibles, tinctures and balms **(83).** The program aims to increase accuracy in product labeling and help consumers to be adequately informed about cannabis products being sold on the market today. The initial phase will focus on potency testing, but a future direction will also include contaminants such as heavy metals and pesticides. It's through the involvement of recognized and well-established standards' organizations like NIS that will help to raise the industry standard for cannabis and hemp testing.

Ways to Reduce Sources of Errors Associated with Sampling and Testing

So let us now take a closer look at some of the most important factors for a testing lab to consider when measuring elemental contaminants in cannabis and hemp, which should help them better understand the major analytical issues and to become more comfortable with working in the ultra-trace element environment **(84).**

- It might seem obvious, but ensure the sample being tested is representative of the batch of cannabis being grown, or the product lot of the cannabinoid being sold or consumed.
- Make sure the cleanliness of the area where the incoming samples are being received. Check for sources of dirt, dust or particulates, which might contain elemental contaminants. In particular avoid metallic surfaces that might be in contact with the samples, such as stainless or galvanized steel.
- ICP-MS offers unparalleled sensitivity and extremely low detection limits, so the instrument should be operated in an area free of environmental contamination. If possible it should be installed in a special positive-pressure; thermostatted room where air can leave the room without circulating back in and fluctuating air temperatures will not negatively impact instrument components, which could lead to signal drift.
- You cannot operate an instrument in a warehouse and expect to get high quality data. Consider installing the instrument in a class 1000 (ISO 6) or class 10,000 (ISO 7) room, which refers to the number of particles of size 0.5 µm or larger per cubic foot of air. As a point of reference, the semiconductor industry (which is typically chasing zero elemental contaminants in its computer chips) will usually install their instruments in a class 10 (ISO 4) or 100 (ISO 5) room. Note: ISO clean room classifications are shown in parenthesis **(85).**
- Avoid metallic grinding equipment in preparing the sample for digestion and analysis, because it could potentially end up in the sample solution you are presenting to the instrument. Cryogenic grinding equipment is very useful, but ensure that polymer-based internal components are used.
- Personal use of jewelry, cosmetics, lotions, perfumes and shampoos can have a negative impact on the analysis as they are all have various elemental components including nickel, chromium, silver, gold, platinum, zinc, silicon, titanium, iron, cadmium.
- An operator that smokes (tobacco or cannabis products) will elevate the background level, particularly for an element like cadmium.
- Sample digestion can be a major source of contamination from used microwave vials, containers and vessels. Have a robust acid cleaning process that ensures your reagent blanks are clean and free of elemental contaminants (a commercial acid cleaning system for vessels, beakers and volumetric ware is an excellent investment).
- All analytical reagents, chemicals, acids, and deionized water should be ultra-high purity grade.
- Use multielement calibration standards designed for use with ICP-MS and not ones made for AA or ICP-OES, which are typically of lower purity
- When carrying out the measurement of heavy metals using the instrumental technique, it is important to adopt a robust quality assurance program (QAP) based on recognized quality management systems such as ISO, and GLP which utilize official mandated methods defined by federal agencies such as FDA, EPA, NIST and USDA or consensus methods put out by independent standards' organizations like ASTM, AOAC, USP AHPA and Emerald. This QAP aspect is absolutely critical, because without currently having a choice of certified reference materials of cannabis and cannabis extracts to validate the accuracy of the method, the analytical procedures must involve recognized spike recovery procedures where all standards, blanks and samples are spiked with known concentrations based on the regulated maximum limits for the cannabis product to ensure that no false positive or negative results are generated.
- One such recognized method to follow is outlined in the validation protocols defined in USP Chapter 233 which should be a critical component of all heavy metal testing of cannabis products. Meeting these performance requirements described in these tests must be demonstrated experimentally using an appropriate system suitability procedure and reference materials to demonstrate detectability, accuracy, specificity, stability, ruggedness and drift. The suitability of the method, known as the J-value validation protocol must be determined by conducting studies with the material under test supplemented/spiked with known concentrations of each target element of interest at the appropriate acceptance limit concentration. It should also be emphasized that the materials under test must be spiked before any sample preparation steps are performed. This spike recovery procedure is a very important part of validating the method and the generated data has to be shown to the regulatory agency as proof that the analysis has

been carried out correctly.

- Understand the errors and uncertainty involved with your analytical methodology including volumetric glassware, analytical balances, calibration standards, reference materials, sample preparation procedures and precision/repeatability of the instrumental technique used.
- Routine maintenance of the instrument components (sample introduction parts, cones, ion optics etc.) should be carried out on a regular basis to minimize contamination from the sample matrices.
- If you are tasked with characterizing cannabis vaping pens, get familiar with methodology for measuring toxic metals in vaping liquids, as well as determining toxic metals in the aerosol being delivered to the consumer, because the requirements are very different, It is relatively straight forward to aspirate the liquid into the spectrochemical measuring technique, using a conventional sample introduction system and quantitate the elemental contaminants, as long as suitable calibration standards are used. However, how do you aspirate an aerosol from the vaping device directly into the instrument and be able to quantitate each puff/inhalation unless you have specialized sampling tools and/or smoking/vaping machines. This becomes very challenging unless you have had experience in carrying out this type of analysis. However, Halstead and Gray and co-workers have recently published two very pertinent papers, which measure a suite of toxic metals in both liquids and aerosols in nicotine-based electronic cigarettes **(86, 87)**. And finally, the author of this white paper has just published a series of articles, which focuses on the new Colorado regulations for measuring heavy metals in vaping aerosols, which will be implemented in January, 2022 **(88)**.

Real-world Detection Capability

It's important to understand all the practical real-world detection capability of the entire analytical methodology and in particular the potential sources of contamination that can impact the limit of quantitation (LOQ), some of which are outlined below:

- Cleanliness of sample preparation test area and equipment
- Laboratory dust/dirt of unknown origin (eg. old Pb-based paint)
- Cleanliness of sample digestion procedure
- Quality of the deionized water
- Purity of analytical reagents, acids and solvents
- Impurities in laboratory glassware and plastic vessels/containers
- Purity of the plastic tubing used in delivering the sample to the instrument
- Contaminants from the analyst, including clothing, cosmetics, lotions, perfumes, shampoo, jewelry, smoke

A Few Words for Regulators

Finally, I'd like to offer a word of advice for regulators who are tasked with inspecting testing labs for heavy metals analysis. You should become familiar with analytical procedures described in USP Chapter 233 and, in particular, the strict validation protocols described in the chapter. These protocols, which are based on strict spike recoveries have been developed and tested by the USP over the past 20 years. They are the very essence of the FDA inspection process of pharmaceutical labs to ensure the data generated is of the highest quality. If a lab is not carrying out these procedures thoroughly and correctly, there is a very good chance the sample data will be flawed.

However, providing data for regulatory inspection and product release documentation in any industry is only part of the challenge facing the modern analytical laboratory. For example in the pharmaceutical industry, external inspections of facilities and procedures means that a great deal of infrastructure is required to support the actual analytical operation including (but not limited to) people, equipment specifications, GLP capability, lab facilities, SOPs, validation and data management. The pharmaceutical analytical laboratory has taken this aspect very seriously, because they are a part of a highly regulated industry. It is unlikely that cannabis state regulators will have the necessary background and experience to carry out this kind of detailed inspection of cannabis testing labs. However, it is a given that when the FDA eventually comes around, they will know exactly what they are looking for and will leave no stone unturned, having had a number of years of experience of regulating the pharmaceutical industry. My previous publication, “Measuring Elemental Impurities in Pharmaceuticals” covers this in greater detail **(89)** but an excellent resource for getting ready for an FDA inspection can also be found in these references **(90, 91).**

Note: There have recently been a number of public announcements from federal and other standards organizations on regulating heavy metals in cannabis and hemp, which are in various stages of publication. Please check out these references for their current positions): FDA **(92),** EPA **(93),** USDA **(94),** NIST **(95),** USP **(96),** AHPA **(97),** ASTM **(98),** and AOAC **(99).**

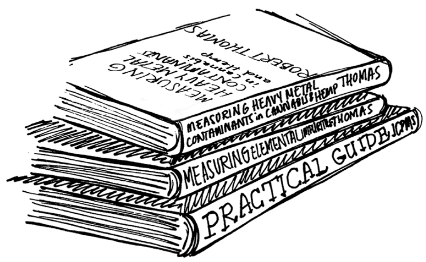
Final Thoughts

Our environment has been severely polluted by heavy metals, which has compromised the ability of our natural ecosystems to foster life and render its intrinsic values. Heavy metals are known to be naturally occurring compounds, but anthropogenic activities introduce them in extremely large quantities into our agricultural ecosystems. Nowhere is this more evident than in the delicate balance of growing and cannabis and hemp for commercial, medicinal, and recreational uses. Unfortunately, the demand for cannabinoid-based products is moving so fast that the scientific community is not keeping up with it; whether it’s the testing of the products to make sure they are safe for human consumption, or the medical research required to understanding the biochemistry that is fundamental to treating a particular disease or ailment.

The industry is both exciting and chaotic at the same time, but, because of its unparalleled growth, there appears to be very little incentive to bring in sensible regulations. There clearly needs to be a more comprehensive suite of elemental contaminants tested and to set the toxicological maximum limits on based on the manner and the quantity that cannabis products are consumed. For that reason, I firmly believe that researchers who are trying to raise the bar now will be recognized when the FDA eventually starts to regulate the industry. However, in the meantime, I’m firmly committed to educating state regulators to better understand the potential sources of heavy metals in cannabis and hemp and to helping testing labs improve the quality of their data **(100).**

Further Reading

1. Marijuana Policy by State: https://www.mpp.org/states/



2. United States Pharmacopeia General Chapter <231> Heavy Metals Test in USP National Formulary (NF), https://www.usp.org/chemical-medicines/elemental-impurities-updates
3. ICH Guideline Q3D on Elemental Impurities: Step 5, European Medicine Agency Website: https://www.ema.europa.eu/en/documents/scientific-guideline/international-conference-harmonisation-technical-requirements-registration-pharmaceuticals-human-use_en-13.pdf)
4. United States Pharmacopeia General Chapter <232> Elemental Impurities – Limits: First Supplement to USP 40–NF 35, 2017, https://www.usp.org/chemical-medicines/elemental-impurities-updates
5. United States Pharmacopeia General Chapter <233> Elemental Impurities – Procedures: Second Supplement to USP 38–NF 33, 2015, https://www.usp.org/chemical-medicines/elemental-impurities-updates
6. From Laboratory Experiments to Large Scale Application – An Example of the Phytoremediation of Radionuclides, P. Soudek et.al. Advanced Science and Technology for Biological Decontamination of Sites Affected by Chemical and Radiological Nuclear Agents, pp 139-158, 2007.
7. Marijuana Toxicity: Heavy Metal Exposure Through State-Sponsored Access to “la Fee Verte”, D. Gauvin et.al., Pharmaceutical Reg Affairs, 7:1, 2018
8. Measuring Elemental Impurities in Pharmaceuticals: A Practical Guide, R. J. Thomas, CRC Press, Boca Raton. FL, US, 2018, ISBN: 9781138197961
9. Measuring Heavy Metal Contaminants in Cannabis and Hemp: A Practical Guide, R. J. Thomas, CRC Press, Boca Raton, FL, ISBN: 9780367417376, Available September, 2020
10. U.S. Domestic Hemp Production Program, USDA Website; https://www.ams.usda.gov/rules-regulations/hemp
11. The Use of Plants for the Removal of Toxic Metals from Contaminated Soil; M. Lasat, AAAS Report, Washington, DC, 2004
12. Phytoremediation Potential of Hemp (Cannabis sativa L.): Identification and Characterization of Heavy Metals Responsive Genes, R. Ahmad et. al., Volume 44, Issue 2, February 2016, Pages 195-201
13. A Budding Cannabis Cottage-Industry has set the

stage for an Impending Public Health Crisis, Gauvin D.V. et.al., Pharmaceut Reg Affairs Vol 7(1): 199, 2018,
14. Marijuana Toxicity: Heavy Metal Exposure Through State-Sponsored Access to "la Fee Verte", D. Gauvin et.al., Pharmaceutical Reg Affairs, 7:1, 2018
15. 15. Effect of Soil Contamination on Some Heavy Metals Content of Cannabis Sativa, Khan Et.al., J. Chem. Soc. Pak., Vol. 30, No.6, 2008.
16. Environmental Contamination by Heavy Metals; V. Masindi and K. L. Muedi, http://dx.doi.org/10.5772/intechopen.76082
17. Arsenic, Cadmium, and Lead in California Cropland Soils: Role of Phosphate and Micronutrient Fertilizers, W. Chen et. al., Journal of Environmental Quality 37(2):689-95, March 2008
18. Extraction of pharmaceutically active components from plant materials, US Patent Number, US7344736B2, Inventors, B. Whittle, C. A. Hill, I. R. Flockhart, D. V. Downs, P. Gibson, G. W. Wheatley, GW Pharmaceuticals, 2008
19. Metal Concentrations in e-Cigarette Liquid and Aerosol Samples: The Contribution of Metallic Coils. P. Olmedo et. al., Environmental Health Perspectives, February, Feb 21;126 (2), 2018.
20. Rhizosphere engineering: Enhancing sustainable plant ecosystem productivity, A.H. Ahkami et. al., Rhizosphere, Volume 3, Part 2, June 2017, Pages 233-243, 2017, https://doi.org/10.1016/j.rhisph.2017.04.012
21. Back to the Root—The Role of Botany and Plant Physiology in Cannabis Testing, Part I: Understanding Mechanisms of Heavy Metal Uptake in Plants, G. Bode, Cannabis Science and Technology, Vol 3, No 2, March 2020
22. Heavy Metals in Soils: Trace Metals and Metalloids in Soils and their Bioavailability; Editor, B. J. Alloway, Department of Soil Science, University of Reading, UK; Springer Science, Dordrecht, 2013, ISBN: 978-94-007-4469-1
23. Single-Particle ICP-MS: A Key Analytical Technique for Characterizing Nanoparticles; C. Stephan, R. Thomas, Spectroscopy Magazine, 32(3) March 2017
24. Using Biochar for Remediation of Soils Contaminated with Heavy Metals and Organic Pollutants; X Zhang et. al., Environmental Science Pollution Research, DOI 10.1007/s11356-013-1659-0, Springer Verlag, 2013.
25. The Impact of Illegal Artisanal Gold Mining on the Peruvian Amazon: Benefits of Taking a Direct Mercury Analyzer into the Rain Forest to Monitor Mercury Contamination, R. J. Thomas, AP Column, Spectroscopy Magazine, March, 2019, Volume 34, Issue 2, pg 22–32 http://www.spectroscopyonline.com/impact-illegal-artisanal-gold-mining-peruvian-amazon-benefits-taking-direct-mercury-analyzer-rain-fo
26. Global mercury emissions from gold and silver mining, L.D. Lacerda, Water, Air, and Soil Pollution volume 97, 209–221, (1997).
27. Heavy metals and living systems: An overview, R. Singh et. al., Indian J Pharmacol, May-June; 43 (3): 246–253, (2011).
28. Common Radionuclides found at Superfund Sites, The U.S. Environmental Protection Agency (EPA), https://www.epa.gov/radiation/which-radionuclides-are-found-superfund-sites
29. Coal Ash Basics, The U.S. Environmental Protection Agency (EPA), https://www.epa.gov/coalash/coal-ash-basics.
30. Urban children are playing in toxic dirt, Yvette Cabrera, Think Progress, July 12, 2017, https://thinkprogress.org/urban-children-are-playing-in-toxic-dirt-41961957ff23/
31. Mercury and Air Toxics Standards (MATS): Clean Air Act, The U.S. Environmental Protection Agency (EPA), https://www.epa.gov/mats
32. Chromated copper arsenate–treated wood: a potential source of arsenic exposure and toxicity in dermatology, A. Yuntzu-Yen Chen and T. Olsen, Int. J. Women's Dermatol., March; 2(1): 28–30, 2016.
33. Heavy metal pollution from phosphate rock used for the production of fertilizer in Pakistan, T. Mamoud et. al., Microchemical Journal 91(1) · September 2008, https://doi.org/10.1016/j.microc.2008.08.009
34. Role of Nickel in Plant Culture, T. Buechel, October, 2018, Promix Website, https://www.pthorticulture.com/en/training-center/role-of-nickel-in-plant-culture/
35. Marijuana Growing Forum, https://www.marijuanagrowing.com/showthread.php?27382-Silica-to-increase-the-yield-of-cannabis.
36. Toxicity of formulants and heavy metals in glyphosate-based herbicides and other pesticides, N. Defarge, J. Spiroux de Vendômoisb, G. E. Séralinia, Toxicology Reports Volume 5, 2018, 56-163, https://doi.org/10.1016/j.toxrep.2017.12.025
37. Hexavalent Chromium, The National Institute for Occupational Safety and Health (NIOSH), https://www.cdc.gov/niosh/topics/hexchrom/default.html.
38. America's Clean Water Crisis Goes Far Beyond Flint. There's No Relief in Sight. J. Moorland, Time Magazine, Feb 20, 2020, https://time.com/longform/clean-water-access-united-states/
39. Flint Drinking Water Response, EPA Continues to Oversee State and City Action to Protect Public Health, The U.S. Environmental Protection Agency (EPA), https://www.epa.gov/flint.
40. Learn about Lead: Factsheet about the hazards of lead paint, The U.S. Environmental Protection Agency (EPA), https://www.epa.gov/lead/learn-about-lead.
41. Influence of Flue Gas Desulfurization Gypsum Amendments on Heavy Metal Distribution in

Reclaimed Sodic Soils, Q. Chan et. al., Environ Eng Sci. 2015 Jun 1; 32(6): 470–478., Environ Eng Sci. 2015 Jun 1; 32(6): 470–478.
42. China: Toxic trails from metal production harms health of poor communities amid soaring global demand for gadgets; G. Shih, Washington Post, January 5, 2020, https://www.business-humanrights.org/en/china-toxic-trails-from-metal-production-harms-health-of-poor-communities-amid-soaring-global-demand-for-gadgets
43. China Cashes in on the Cannabis Boom, New York Times editorial, S. Meyers, May 4, 2019, https://www.nytimes.com/2019/05/04/world/asia/china-cannabis-cbd.html
44. United States Pharmacopeia General Chapter <232> Elemental Impurities – Limits: First Supplement to USP 40–NF 35, 2017, https://www.usp.org/chemical-medicines/elemental-impurities-updates
45. United States Pharmacopeia General Chapter <233> Elemental Impurities – Procedures: Second Supplement to USP 38–NF 33, 2015, https://www.usp.org/chemical-medicines/elemental-impurities-updates
46. ICH Website: http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html (Q3D)
47. Cannabis Concentrates: Differences Between Microwave and Ultrasonic Assisted Extraction, A. Mayfield, Extraction Magazine, March 22, 2018, https://extractionmagazine.com/2018/03/22/cannabis-concentrates-differences-between-microwave-and-ultrasonic-assisted-extraction/
48. Advances in Cannabis Extraction, A Beadle, Analytical Cannabis, June 15, 2019, https://www.analyticalcannabis.com/articles/advances-in-cannabis-extraction-techniques-311772
49. New Extraction Technologies Lining Up to Be Game-Changers, D. Hodes, Cannabis Science and Technology, Vol 3, Issue 4, May, 2020, https://www.cannabissciencetech.com/extraction/new-extraction-technologies-lining-be-game-changers
50. Effect of Soil Contamination on Some Heavy Metals Content of Cannabis Sativa, Khan Et.al., J. Chem. Soc. Pak., Vol. 30, No.6, 2008.
51. Back to the Root—The Role of Botany and Plant Physiology in Cannabis Testing, Part I: Understanding Mechanisms of Heavy Metal Uptake in Plants, G. Bode, Cannabis Science and Technology, Vol 3, No 2, March 2020, https://www.cannabissciencetech.com/metals/back-root-role-botany-and-plant-physiology-cannabis-testing-part-i-understanding-mechanisms-heavy
52. The Presence and Transmission of Heavy Metals in Plant Fertilizers, L. Macri, Maximum Yield, September 1, 2016, https://www.maximumyield.com/the-presence- and-transmission-of-heavy-metals-in-plant-fertilizers/2/2640
53. Mercury Contamination of Aquatic Ecosystems, USGS Fact sheet, Fact Sheet 216-95, https://pubs.usgs.gov/fs/1995/fs216-95/
54. Mercury in the Food Chain, Health Canada, https://www.canada.ca/en/environment-climate-change/services/pollutants/mercury-environment/health-concerns/food-chain.html
55. CBD Oil Product Recalled Due to High Lead Levels, FDA Announces, L. Bear-McGuinness, Analytical Cannabis, May 20, 2020; https://www.analyticalcannabis.com/news/cbd-oil-product-recalled-due-to-high-lead-levels-fda-announces-312418
56. Lawsuit over heavy metals in CBD products raises more questions than answers, E. Watson, Food Navigator, May 11, 2020, https://www.foodnavigator-usa.com/Article/2020/05/11/Lawsuit-over-heavy-metals-in-CBD-products-raises-more-questions-than-answers-says-attorney-Hemp-Authority-seal-gains-traction#
57. Marijuana contaminated with heavy metals, pesticide recalled in testing lab investigation, A. Biolchini, Michigan live, August, 30, 2019, https://www.mlive.com/news/2019/08/marijuana-contaminated-with-heavy-metals-pesticide-recalled-in-testing-lab-investigation.html
58. Ohio medical marijuana program issues first mandatory product recall, L. Hancock, Cleveland Online, November 15, 2019, https://www.cleveland.com/open/2019/12/state-regulators-issue-second-mandatory-recall-of-ohio-medical-marijuana-product.html
59. California Testing Lab Falsification Might Cause Huge Cannabis Product Recall, December 4, 2018, EcoWaste, https://ecowasteservices.com/california-cannabis-testing-lab-falsification-recall
60. Maryland cannabis regulators warn of lead contamination risk as they expand tests for heavy metals D. Donovan, The Baltimore Sun, June 4, 2019, https://www.baltimoresun.com/health/marijuana/bs-md-cannabis-heavy-metals-20190605-story.html
61. Hawaiian Cannabis Vape Cartridges Are Contaminated With Ethanol and Lead, Tests Find, L. Bear-McGuiness, June 9, 220, Analytical Cannabis, https://www.analyticalcannabis.com/news/hawaiian-cannabis-vape-cartridges-are-contaminated-with-ethanol-and-lead-tests-find-312447
62. Extraction of Pharmaceutically Active Components from Plant Materials, G. Whittle et. al., United States Patent, Number: 7,344.736, March 18, 2008, https://patentimages.storage.googleapis.com/8b/d5/bb/9c377f6598f6a2/US7344736.pdf
63. Cannabis contaminants: sources, distribution, human toxicity and pharmacologic effects, L. Dryburgh

et.al., Br J Clin Pharmacol 2018 Nov; 84(11): 2468–2476, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6177718/

64. Cannabis Contaminants: Regulating Solvents, Microbes, and Metals in Legal Weed, N. Seltenrich, Environmental Health Perspectives, 20 August 2019, https://doi.org/10.1289/EHP5785
65. America's Clean Water Crisis Goes Far Beyond Flint. There's No Relief in Sight. J. Moorland, Time Magazine, Feb 20, 2020, https://time.com/longform/clean-water-access-united-states/
66. Metal Concentrations in e-Cigarette Liquid and Aerosol Samples: The Contribution of Metallic Coils. P. Olmedo et. al., Environmental Health Perspectives, February, Feb 21; 126 (2), 2018.
67. Heavy Metals Contamination: Is Cannabis Packaging to Blame? R. Newman, Analytical Cannabis, Feb 20, 2020, https://www.analyticalcannabis.com/articles/heavy-metals-contamination-is-cannabis-packaging-to-blame-312246
68. Facing the Problem of Dietary-Supplement Heavy-Metal Contamination: How to Take Responsible Action, R. Liva, Integrative Medicine, Vol. 6, No. 3, Jun/Jul 2007, http://www.imjournal.com/resources/web_pdfs/0607_liva.pdf
69. 69. Journey of a Tainted Vape Cartridge: from China's Labs to Your Lungs, D. Downs, D. Howard, B. Barcott, Leafly, September 24, 2019, https://www.leafly.com/news/politics/vape-pen-injury- supply-chain-investigation-leafly
70. Toxic Metal Concentrations in Mainstream Smoke from Cigarettes Available in the USA, R. Pappas et al. al. Journal of Analytical Toxicology; 38:204–211, 2014
71. Analysis of Heavy Metals in Cigarette Tobacco, J. Pariati, Journal of Medical Discovery, March 2017.
72. Toxic Metal Concentrations in Mainstream Smoke from Cigarettes Available in the USA, R. Pappas et al. Journal of Analytical Toxicology; 38:204–211, 2014
73. Toxic Metals Found in E-Cigarette Vapor, A Moses, WebMD, Feb 26, 2018, https://www.webmd.com/smoking-cessation/news/20180226/toxic-metals-found-in-e-cigarette-vapor#1
74. ***The State of Cannabis Research Legislation in 2020*** by A.J. Zarrabi, et.al., .New England Journal of Medicine, HHS Public Access, June 16, 2020, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7302267/pdf/nihms-1597843.pdf
75. Holding Data to a Higher Standard, Part II: When Every Peak Counts-A Practical Guide to Reducing Contamination and Eliminating Error in the Analytical Laboratory, P. Atkins, Cannabis Science and Technology, 1 (4), 40–49 (2018), https://www.cannabissciencetech.com/view/holding-data-higher-standard-part-ii-when-every-peak-counts-practical-guide-reducing-contamination
76. United States Pharmacopeia General Chapter <231> Heavy Metals Test in USP National Formulary (NF), https://www.usp.org/chemical-medicines/elemental-impurities-updates
77. United States Pharmacopeia General Chapter <232> Elemental Impurities – Limits: First Supplement to USP 40–NF 35, 2017, https://www.usp.org/chemical-medicines/elemental-impurities-updates
78. United States Pharmacopeia General Chapter <233> Elemental Impurities – Procedures: Second Supplement to USP 38–NF 33, 2015, Pharmacopeia Online, https://www.usp.org/chemical-medicines/elemental-impurities-updates
79. USP General Chapter <730> Use of Plasma Spectrochemistry for Pharmaceutical Analysis, Pharmacopeia Online, http://www.uspbpep.com/usp31/v31261/usp31nf26s1_c730.asp
80. USP General Chapter <1225> for Validation of Compendial Procedures , Pharmacopeia Online, http://www.uspbpep.com/usp29/v29240/usp29nf24s0_c1225.html
81. ICH Q3D Step 5 Guidelines, ICH Website: http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html (Q3D)
82. Emerald Proficiency Test, Emerald Scientific, https://pt.emeraldscientific.com/
83. Feds Launch Cannabis Testing Program To Help Consumers Know What They're Buying, K. Jarger, Marijuana Moment, July 22, 2020; https://www.marijuanamoment.net/feds-launch-cannabis-testing-program-to-help-consumers-know-what-theyre-buying/
84. Clean Chemistry for Elemental Impurities Analysis of Pharmaceuticals in Compliance with USP Chapter <232>, Chunguang Jin, AAPS PharmSciTech, 17, 1141–1149 (2016); https://link.springer.com/article/10.1208/s12249-015-0452-4
85. ISO Clean Room Particle Count Classifications, https://www.mecart-cleanrooms.com/learning-center/cleanroom-classifications-iso-8-iso-7-iso-6-iso-5/
86. Analysis of Toxic Metals in Liquid from Electronic Cigarettes; N. Gray et. al., Int. Journal Environ Res Public Health. 2019 Nov; 16(22): 4450.
87. Analysis of Toxic Metals in Electronic Cigarette Aerosols Using a Novel Trap Design, M. Halstead et.al. Journal of Analytical Toxicology, 2019; 001: 1-7
88. New Colorado Regulations for the Measurement of Heavy Metals in Cannabis Vaping Aerosols, A Realistic and Practical Assessment R.J. Thomas, Analytical Cannabis, November 16, 2020, https://www.analyticalcannabis.com/articles/new-colorado-regulations-for-the-measurement-of-heavy-metals-in-cannabis-vaping-aerosols-a-312812
89. Measuring Elemental Impurities in Pharmaceuticals: A

Practical Guide, R. J. Thomas, CRC Press, Boca Raton. FL, US, 2018, ISBN: 9781138197961, https://www.routledge.com/Measuring-Elemental-Impurities-in-Pharmaceuticals-A-Practical-Guide/Thomas/p/book/9781138197961?utm_source=crcpress.com&utm_medium=referral

90. The Food and Drug Newsletter, 2013, https://www.fdanews.com/ext/resources/files/The_Food_And_Drug_Letter/2013/Inspection-Readiness-ExecSeries.pdf
91. Q3D Elemental Impurities Guidance for Industry, U. S. Department of Health and Human Services, FDA, Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), March 2020, http://www.fda.gov/regulatory-information/search-fda-guidance-documents/q3dr1-elemental-impurities
92. FDA Regulation of Cannabis and Cannabis-Derived Products, Including Cannabidiol (CBD), https://www.fda.gov/news-events/public-health-focus/fda-regulation-cannabis-and-cannabis-derived-products-including-cannabidiol-cbd
93. EPA Seeks Public Comment on Pesticide Applications for Hemp, https://www.epa.gov/newsreleases/epa-seeks-public-comment-pesticide-applications-hemp
94. 94. U.S. Domestic Hemp Production Program, USDA Website; https://www.ams.usda.gov/rules-regulations/hemp
95. 95. NIST Tools for Cannabis Laboratory Quality Assurance, https://www.nist.gov/programs-projects/nist-tools-cannabis-laboratory-quality-assurance
96. Cannabis Inflorescence for Medical Purposes: USP Considerations for Quality Attributes, N. D. Sarma, et.al., ACS Journal of Natural Products, 2020 83 (4), 1334-1351, https://pubs.acs.org/action/showCitFormats?doi=10.1021/acs.jnatprod.9b01200&ref=pdf
97. AHPA Cannabis Committee, http://www.ahpa.org/AboutUs/Committees/CannabisCommittee.aspx
98. ASTM Committee D37 on Cannabis, https://www.astm.org/COMMITTEE/D37.htm
99. AOAC Cannabis Analytical Science Program https://www.aoac.org/scientific-solutions/casp/
100. Educational Workshop on Heavy Metals in Cannabis by ICP-MS Video Recording of the Presentations, R. Thomas, Nov 10, 2020, https://www.analyticalcannabis.com/articles/workshop-on-heavy-metals-in-cannabis-by-icp-ms-now-available-to-view-312735

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