Evaluation of methods used to sample hemp for regulatory compliance testing

David R. Gang and Anna Berim
Tissue Imaging, Metabolomics and Proteomics Laboratory
Institute of Biological Chemistry
Washington State University
Pullman, WA 99164-6340

As outlined in the "Establishment of a Domestic Hemp Production Program; Comment Period Reopened" document, multiple comments made during the previous open comment period for the Interim Final Rule (IFR) raised clear questions regarding the "uncertainty of sampling" of hemp samples being analyzed to determine field compliance with legal THC levels for hemp (being defined as ≤0.30% THC by dry weight).

In order to address this concern and to better understand how best to sample hemp plants to test for total THC (delta-9-tetrahydrocannabinol) potency compliance, researchers at Washington State University (led by Professor David R. Gang, Fellow in the Institute of Biological Chemistry and the Assistant Director of WSU's Agricultural Research Center and the CAHNRS Office of Research in the College of Agricultural, Human and Natural Resource Sciences in collaboration with Dr. Anna Berim, senior Research Associate within the Institute of Biological Chemistry, Washington State University) were asked by the Washington State Department of Agriculture (which oversees Washington State's Industrial Hemp program) to compare the new sampling protocol that was implemented by the USDA for 2020 to the method approved for use in Washington State in 2019.

These researchers performed a series of experiments to compare the sampling methods to each other (and to alternative methods) with the aim of determining how consistent (or not) the methods are relative to each other in being able to accurately and reliably determine <u>total THC levels</u> within hemp plants in the field.

Total THC levels are determined by adding free THC levels and THCA levels (Tetrahydrocannanolic acid, corrected for the difference in mass of the two compounds) using the formula: Total THC = THC + THCA*0.877, per the IFR. This formula takes into account the difference in mass of THC (formula $C_{21}H_{30}O_2$, 314.469 g·mol^{-1}) and THCA (formula $C_{22}H_{30}O_4$, 358.478 g·mol^{-1}); with 314.469/358.478 = 0.877234. Rounding down the multiplication factor to 0.877 leads to a very slight (0.0266%) underestimation of the total THC contribution by THCA in the calculation. This difference is well below anything significant compared to the chemical level variability in the hemp plants being analyzed or the accuracy of high-end, state of the art analytical instruments available today, as outlined below. The calculations used in this analysis do not take into account questions raised in the original comments related to the "Liquid Chromatography Factor, 0.877" regarding potential loss of THCA to thermal degradation instead of being converted at a 100% efficiency to THC. This report therefore recognizes that levels of actual "total THC" that is physiologically relevant could be lower than determined using the calculation-based methods employed herein. That fact in no way changes the conclusions of this report with regards to the adequacy, reproducibility and robustness of different sampling methods to be employed when determining THC levels in hemp fields.

Evaluation of methods used to sample hemp for regulatory compliance testing

Four different sampling methods to be compared.

2020 Top 1/3 Plant Sampling Method: This is the current USDA-approved sampling method employed in 2020, with each field sampled at 15 days preharvest, and the top 1/3 of representative plants in the field being used for analysis. Briefly, "representative field samples" are collected and then pooled to form a combined sample per field, which is then dried. A "representative" subfraction of the dried plant material is ground either to a dry powder or directly in a solvent (depending on the specific implementation in the performing laboratory), and is then extracted by a solvent (typically methanol) and then analyzed by a method (either gas chromatography- or liquid chromatography-based) that measures total THC levels (either post-decarboxylation or combining data from free THC and THCA) to determine the "total THC" level for the field. During the implementation of this sampling process, a number of plants are sampled per hemp field, wherein a single branch from the top 1/3 of each plant is collected and added to a pool of branches per field, with the number of plants sampled being determined by the size of the field. The larger the field, the larger the number of branches sampled, but a minimum of 10 branches are typically sampled. These branches are then dried at a temperature low enough to efficiently remove moisture while maintaining THCA integrity. It is well-known that heating THCA to temperatures in excess of 40 °C (104 °F) leads to decarboxylation of THCA to form free THC. It is for that reason that "total THC" levels are measured and not just free THC, because processes such as heating (as occurs in smoking) converts THCA to THC, thereby leading to potential psychoactive compound levels that would exceed the legal limit as defined for hemp in the 2018 Farm Bill. Once the branches are dried, the dried flower and leaf tissues are removed from each branch and representative sample is taken from the dried plant material per branch (including leaf, flower and stem), then mixed into a large pooled sample containing dried plant material from each branch. From this pooled sample, a representative sample is taken and extracted and then analyzed for cannabinoid profile, with Total THC levels being determined using the formula shown above. It should be clear from the above written description, that the word "representative" is used multiple times in the description of how to perform the sampling and then extraction of those samples to generate a number used to determine if a particular hemp field is compliant or not regarding THC levels. A "representative" tissue sample from "representative" branches from the top 1/3 of "representative" plants is what gets extracted.

The way this procedure is typically implemented, just 0.2 g of plant material is actually extracted for the compliance analysis per hemp field. That is a very small amount of tissue relative to a large field, and leaves open the issue of potential stochastic sampling error. If the field contains plants that are not completely uniform in their THC levels relative to each other, it is possible that this small subsample in any given analysis could over-represent plants that have higher levels of THC, thereby leading to failure of the field. On the other hand, equally possible, that analysis could over-represent plants that have lower levels of THC, leading to passing the field. This issue is addressed below in more detail. Indeed, as the results presented below clearly demonstrate, we found that such sampling errors are not only possible, but the likely result in any given test. In other words, the most likely result of a sampling test is an inaccurate assessment of the total THC levels based on the method used to sample the plants in the field and then prepare them for extraction.

After extraction, as suggested by the comments provided in the previous open comment period, the results are very consistent. Indeed, the method used to measure the total THC levels in the extracts was found to be extremely reproducible in the hands of the WSU researchers. During the analyses performed at WSU, the researchers included a series of quality control (QC) samples, all being aliquots of the same exact mixture of cannabinoid standards and run periodically throughout the analyses that were performed over many days. Despite the fact that these QC samples were analyzed on different days, the WSU researchers found that the coefficient of variance of the results for THC levels in those QC samples was less than 1% (0.581% in fact). Clearly, the methods for measuring THC in extracts are highly

robust and reproducible. The question then arises regarding the same for the sampling procedure, as described in more detail below. More detailed methods follow in the attached document: "Detailed Methods for Comparison of Sampling Methods for THC Potency Testing of Hemp" by David R. Gang and Anna Berim.

2019 Whole Plant Remediated Sampling Method: This method was employed in Washington State in 2019 (WSDA approved method) and collected representative "whole plant remediated" samples at 30 days preharvest, which included taking leaf, flower, and stem samples from regions of the whole plant (bottom 1/3, middle 1/3, top 1/3). In this sampling method, the leaf and flower tissues are dried, crushed separately and a "representative sample" of the dried plant material is then ground separately by tissue type and fractions thereof taken at a ratio of 37:30 and combined, or the tissues are mixed at that ratio prior to grinding. The ground sample is then extracted and analyzed as for the 2020 Top 1/3 Plant Sampling Method described above. This ratio (37:30) represents the fraction of the hemp plant at 30 days to 15 days preharvest that is leaf tissue or flower tissue, respectively, with the remaining 33% of the tissue of a hemp plant consisting of stem/stalk tissue. Because only flower and leaf material is extracted using this method, the sample is "remediated" by multiplying the determined total THC level by 0.67, thereby taking into account the mass of the plant made up by stem/stalk. The same issues described for the 2020 Top 1/3 Plant Sampling Method regarding potential stochastic sampling bias due to small amount of tissue per field being actually extracted holds for this sampling method. Again, a "representative" tissue sample from "representative" plants is what gets extracted. Again, that representative tissue sample is a very small sample size in actual fact (~0.2 – 0.25 g as typically implemented).

2020 Whole Plant Remediated Sampling Method: This method follows the procedure for the 2019 Top 1/3 Plant Sampling Method, but collects the plant material to be analyzed at 15 days preharvest instead of at 30 days preharvest. The reason for including this sampling procedure was to enable robust comparison of the sampling methods, to eliminate any potential bias that could be introduced by sampling plants 2 weeks apart and then comparing their THC levels.

2020 Top 1/3 Plant Individual Plant Sampling Method: This method was implemented by the WSU researchers to determine what level of cannabinoid variability actually existed in the fields that were used for the analysis described in this report. Plant branches were collected and dried as described above for the 2020 Top 1/3 Plant Sampling Method. Indeed, for the analysis performed by WSU researchers in 2020, the same exact branches were used for the individual plant analysis that were used following the standard USDA method described above. However, prior to mixing the dried plant material into a large pooled sample, individual samples per plant (half of each branch) were separated out to be analyzed separately by plant branch, thereby enabling the analysis and comparison of individual plants and determination of the variation of cannabinoid levels present in the field. In total at least 10 plants per field were thus individually sampled and THC (and other cannabinoid) levels measured.

Implementation of Sampling Procedure in Washington State in 2020.

The WSU researchers collected all field samples required to perform the analysis described in this report. Indeed, the same individual, Professor David Gang, performed all sampling in the field. And a single researcher (a senior Research Associate in the Institute of Biological Chemistry at WSU) performed all extractions and chemical analyses in the lab. The reason for involving just these two individuals in the analysis at WSU was to ensure that no investigator bias would be introduced in the analysis.

Evaluation of methods used to sample hemp for regulatory compliance testing

Samples were collected from 5 separate fields in the eastern half of Washington State, designated in this report by the name of the town that is closest in proximity to the field sites. All were outdoor grows, with plants grown in soil in the field using different cultivation methods (different fertilization regimes, different weed control measures, different plant spacing, etc.). Different hemp varieties were used in these fields, either as single varieties per field or mixed varieties per field, so that results could be extrapolated to other hemp varieties in Washington State, in other words so that robust conclusions regarding the analysis could be drawn that would apply to hemp in general, and not just a single hemp "variety". Herein "variety" means a breeding or cultivar stock that is grown in the field for purposes of hemp production either for research purposes or for ultimate product generation.

Samples for THC potency compliance testing by the WSU researchers were collected from each of those 5 fields on 2 separate occasions (30 days preharvest collection on August 24, 2020 and 15 days preharvest collection on September 7, 2020 [except for the samples collected for 15 day preharvest at the Elk field, which were collected on September 8, 2020) per each sampling protocol as described above, with at least 10 plants per field sampled for the analysis. Samples were placed in the field into labeled paper bags, kept in a large cooler to keep the samples cool and then transported by SUV to a laboratory in the Institute of Biological Chemistry on the Pullman campus of Washington State University, where they were laid out on screens to be dried at <24 °C (<75 °F) in a room with low relative humidity and a light fan breeze blowing over the samples, to prevent any mold formation or rotting of the plant material while drying. Once the samples were dried, they were separated out by tissue type as indicated in the method procedures and partitioned into representative samples to be processed either at WSU or at other labs. Dried samples for each field were thus processed at WSU using the standard extraction procedure implemented in Washington State, and an aliquot of each sample was sent to a separate research lab within Washington State. With 5 fields included in the analysis and 4 separate sampling methods employed, a total of 200 individual samples were dried, ground and analyzed for total THC levels in this method comparison test. These samples were each analyzed by two labs (WSU, second research lab in Washington State) and results of those analyses compared.

Results from Comparison of Sampling Methods

As can be seen in the following tables and charts, the sampling method employed can have a large impact on the results obtained. Note: as described above, the method of analysis after extraction that was used by the WSU researchers and the second research laboratory is very robust and reproducible, with the coefficient of variance (% error) of the technical, analytical method being less than 1% (just 0.581% for WSU) in these analyses. What was observed in the hemp analysis data (see Table 1), on the other hand, was a relative percent error in the analysis that was typically an order of magnitude higher, ranging from 3.2% to 13.8% for the remediated sampling methods to as high as 54% to 67% for the top 1/3 plant based methods. This large variation in results can only be ascribed to the lack of ability of the sampling procedure to generate a consistent, reproducible sample from any given hemp field. Because this error was observed over and over across multiple hemp fields growing different hemp varieties/lines, it is a systemic problem with the sampling procedure and not an artifact of analysis of one particular hemp line.

Table 1. Comparison of Sampling Methods for Total TCH Determination

Total THC (% in sample), Average of 10 samples measured

Field	30d remediated	15d remediated	15d top1/3	15d top1/3_individuals	15d top1/3_HS
Prosser	0.087	0.071	0.176	0.157	0.220
Elk	0.038	0.106	0.343	0.071	0.070
Kennewick-CB	0.067	0.237	0.452	0.435	0.200
Kennewick-YB	0.125	0.187	0.391	0.241	0.240
Mabton	0.027	0.039	0.066	0.058	0.070

Standard Deviations (% in sample)

Field	30d remediated	15d remediated	15d top1/3	15d top1/3_individuals	15d top1/3_HS
Prosser	0.020	0.012	0.186	0.296	0.450
Elk	0.004	0.032	0.213	0.024	0.030
Kennewick-CB	0.014	0.056	0.190	0.766	0.410
Kennewick-YB	0.023	0.081	0.422	0.416	0.510
Mabton	0.003	0.006	0.031	0.048	0.040

Standard Error (% in sample)

Field	30d remediated	15d remediated	15d top1/3	15d top1/3_individuals	15d top1/3_HS
Prosser	0.006	0.004	0.059	0.094	0.142
Elk	0.001	0.010	0.067	0.008	0.009
Kennewick-CB	0.005	0.018	0.060	0.242	0.130
Kennewick-YB	0.007	0.026	0.133	0.132	0.161
Mabton	0.001	0.002	0.010	0.015	0.013

Relative Percent Error (%)

Field	30d remediated	15d remediated	15d top1/3	15d top1/3_individuals	15d top1/3_HS
Prosser	7.2	5.3	33.4	59.6	64.7
Elk	3.2	9.6	19.6	10.7	13.6
Kennewick-CB	6.8	7.5	13.3	55.6	64.8
Kennewick-YB	5.9	13.8	34.1	54.5	67.2
Mabton	3.3	4.7	14.7	26.1	18.1

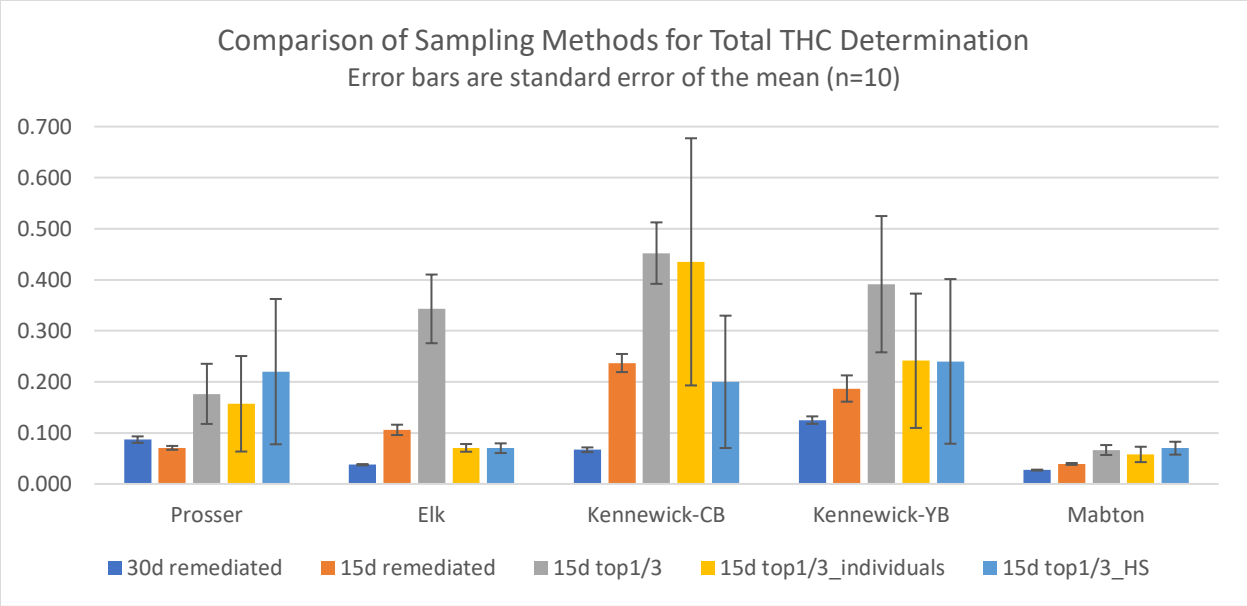

Figure 1. Comparison of total THC levels determined for five different hemp fields when sampled using four different sampling methods and then analyzed at two different analytical laboratories. The 30 day preharvest remediated whole plant sample (30d remediated), the 15 day preharvest remediated whole plant sample (30d remediated), the 15 day top 1/3 plant representative sample (15d top1/3) and the 15 day top 1/3 plant individual plant sample (15d top1/3_individuals) samples were all analyzed in the Institute of Biological Chemistry at Washington State University. A second analysis of the 15 day top 1/3 plant individual plant sample (15d top1/3_HS) samples was performed at a separate research lab in Washington State.

Indeed, when data for individual plants used in this analysis were evaluated closely, it became apparent that THC levels varied significantly from plant to plant, and this was most pronounced in the top 1/3 of the plant samples. For example, in the Kennewick-CB field, THC levels ranged from 0.06% to 2.46% in the top 1/3 plant samples when individual plants were evaluated separately (more than half of the plants had THC levels less than 0.08%, only four exceeded 0.1%, with three of those being “hot” and exceeding 0.3%). In the pooled sample for the 15 day preharvest top 1/3 plant analysis, the THC levels ranged from 0.24% to 0.85%. And, in the whole plant remediated samples for this field, also collected at 15 days preharvest, these levels ranged from 0.16% to 0.31%, with only one sample exceeding 0.3%. If all plants in the field had approximately the same levels of THC, the measured values would have been close to identical based on the robustness of the analytical procedure. If the variation in plant THC levels were to be uniformly distributed across the field, it again would be expected that, on average, the values obtained from a uniform sampling procedure would generate an accurate measure of the field-level THC levels. However, the sampling experiments that were performed indicated that such uniform distribution does not occur at the field level, at least for the hemp lines that were grown in the fields evaluated here. Individual plants with significantly different THC levels than the average for the field can have a large impact on the results obtained for the field. If even a single plant has high THC levels, and that plant happens to be included in the analysis, it can significantly skew the results, especially, apparently, when the top 1/3 plant sampling method is used.

It is obvious from these results that the current USDA method, which analyzes only the top 1/3 of the plant, generates data that is very error-prone and results that likely do not represent the actual THC levels that are present in the hemp plants in the field as a whole. Following the current method, it is thereby possible, indeed likely, that stochastic effects come into play in the analysis and can significantly

affect the outcome. An alternative method, such as using a whole plant remediated sample or testing samples post-harvest is warranted.

Ring test to determine lab-to-lab variability

A ring test experiment was performed to determine what type of variation in analysis could be expected if different analytical labs were to test a given sample. Samples for this experiment were generated using the official 2020 sampling method (2020 Top 1/3 Plant Sampling Method) as described above and sent to analytical labs as would typically be done by the WSDA for a field being compliance tested. Separate commercial labs that are approved to test for the WSDA were included (they were not told that they were involved in this experiment, samples were just sent to them for analysis as any grower would send them, so that their results would not be biased by a desire to compare favorably to other labs). Thus, it was a blind experiment with regards to the commercial labs. In addition, the WSU lab and the other research lab in Washington State that was involved in the other experiments described above also tested the same samples. These samples consisted of plant material collected from the top 1/3 of the plant from at least 10 plants in 5 separate fields (following the standard approved procedure, as described above). The aggregate/pooled sample per field of crushed hemp material was then mixed well and a sub-sample (4 – 5 g of dried plant material consisting of mostly leaf and flower tissue, but also some small stems, as is typically collected for the top 1/3 of the plant sample type) was then sent to each lab for analysis. Thus, a total of 4 measurements (4 labs) were made on samples from 5 separate fields. Results of this analysis are presented in the graphs below.

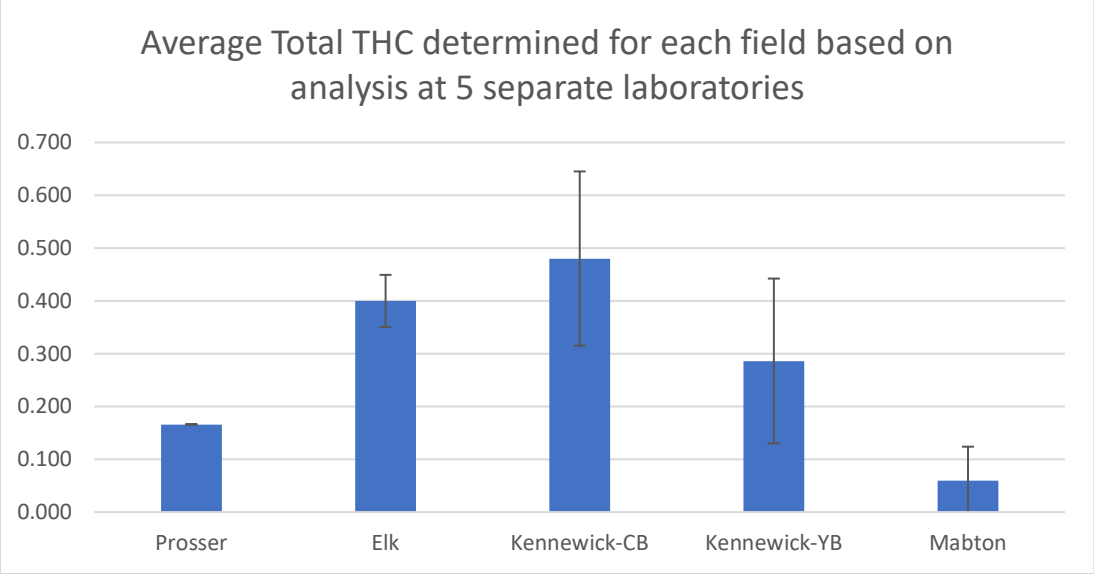

Figure 2. Average Total THC determined for each field included in this analysis, based on analysis performed at 4 separate analytical laboratories. The error bars are standard deviations and help show the variation in the results obtained.

It is important to note that on average, as shown in Figure 2, all fields except Kennewick-CB and Elk would pass compliance if the average of these four labs were taken. What was surprising was that the Elk field would not pass on average, even though all data from the analysis described above comparing the sampling methods clearly showed that the Elk field had, on average, a low total THC level, when alternative methods of sampling were employed. It was the nature of the top 1/3 plant representative sample method, which is very prone to stochastic effects (one small piece of “hot” tissue in the sample

that is taken can significantly impact the result observed), that led to this result in the ring test. When the Elk results were evaluated at the individual plant and individual sample level, it was found that the overall THC levels are low, but a few plants have elevated levels. Over-sampling of the "hot" plant tissue can therefore lead to a non-compliant test result even if the field on average is compliant. The standard deviation in this comparison was relatively high (ranging from 22% – 41%, depending on the field), again indicating that the specific sub-sample that is ultimately selected for extraction and analysis plays a large role in THC level determinations when using the current sampling method.

Table 2. Average Total THC determined for each field based on analysis at 5 separate laboratories

Field	Total THC (%)	Stdev	Percent Deviation
Prosser	0.166	0.049	30%
Elk	0.400	0.165	41%
Kennewick-CB	0.480	0.156	33%
Kennewick-YB	0.286	0.064	22%
Mabton	0.059	0.020	34%

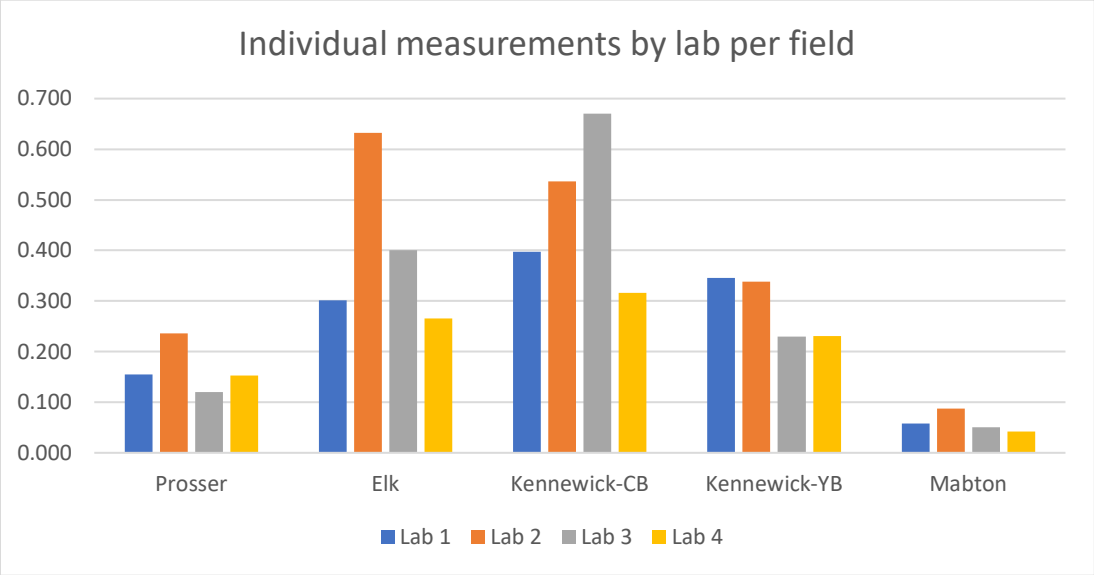

Figure 3. Individual results per lab for compliance testing of the same sample (crushed hemp tissue, not powdered and homogenized).

Overall Conclusions

As described above, significant variation should be expected when analyzing samples collected using the 2020 Top 1/3 Plant Sampling Method (current official USDA method). That was indeed the case for the SAME sample sent to each of 4 labs for analysis. Results obtained back varied by more than 2-fold depending on which laboratory results are compared to each other. This difference was meaningful because the differences led to samples being determined as non-compliant by one lab but compliant by another. Because the sample sent to each lab was from the same pooled sample, and consisted of plant

material that was crushed to less than ¼ inch on average pieces but not ground to a fine powder (that grinding was performed in each lab), the exact sub-sample that was ground in each lab was not the same and therefore small pieces of high THC flower material from a "hot" plant can skew the results obtained for an entire field.

The results of this analysis clearly demonstrate that the current sampling method is inadequate and unable to generate reproducible and robust results, either within a single laboratory and especially across multiple laboratories. Alternative sampling methods (not the top 1/3 plant representative sample method) need to be employed to more accurately determine total THC levels in hemp fields grown for commerce. The whole plant remediated method more accurately reflected what was observed when multiple individual plants were each analyzed separately and then the results from their analysis combined into an average value.

Due to the deadline imposed for commenting, it was impossible to compare post-harvest sample results to the 15 day preharvest or the 30 day preharvest sampling results. Harvesting of hemp in WA state is just happening now, immediately prior to the commenting deadline, and compliance testing results take at least a week to be generated if the testing lab has nothing else to do (which is never the case). The question regarding measurement uncertainty (MU) is still open regarding the current sampling method and needs to be revisited.

Detailed Methods for Comparison of Sampling Methods for THC Potency Testing of Hemp

The Methods outlined in this document were developed and implemented by Prof. David R. Gang and Dr. Anna Berim in the Tissue Imaging, Metabolomics and Proteomics Laboratory, Institute of Biological Chemistry, Washington State University as follows:

We collected samples from 5 fields across central and eastern Washington State to aid in our comparison of sampling procedures for potency testing of hemp:

1. Kennewick field 1 = "Yong×Bern"
2. Kennewick field 2 = "Cherry×Bern"
3. Prosser field = combined "Yong×Bern" (80%) and "Cherry×Bern" (20%)
4. Mabton field = Tricrop
5. Elk field = combined "Yong×Bern" (70%) and "Cherry×Bern" (30%)

We collected 3 separate sample types from each of these fields:

- 30 day preharvest "whole plant remediated sample" (collected 20200824) – 2019 sampling protocol
- 15 day preharvest "whole plant remediated sample" (collected 20200907 – except for Elk field on 20200908) – 2019 sampling protocol, but at 15 days preharvest instead of 30 days
- 15 day preharvest "top 1/3 of plant representative sample" (collected 20200907 – except for Elk field on 20200908) – 2020 sampling protocol at 15 days preharvest

For each of these sample types, at least 10 plants per field were sampled (typically 12 – 13 to ensure that at least 10 plants will be in representative samples if some of the plant tissue becomes "corrupted" (e.g., rots, gets crushed and is no longer useable) prior to drying – we didn't see this problem happen, however). At least 500 g of fresh plant tissue was collected per field using sharp garden clippers, and placed into large thick paper bags (taller and thicker than typical grocery store shopping bags), with each bag labeled with field location, plant genetics, and date of collection, and then each bag was placed into a cooler in the collection vehicle to keep from getting too hot on the collection days.

Samples were taken to WSU's Pullman campus, to the Tissue Imaging, Metabolomics and Proteomics Laboratory (TIMPL) within the Institute of Biological Chemistry, for sample drying and then sample processing and downstream chemical analysis both at TIMPL and at Hops Extract Corporation of America (Hopsteiner) in Yakima, WA.

Processing and drying of whole plant remediated samples (2019 method):

Samples were collected in the field into large paper bags as outlined above, with pieces of at least 10 plants collected such that stems of various sizes, older large leaves, young small leaves, flowers, etc., are all collected and placed into the bag. Due to the nature of the sampling, it was not possible to connect any given piece of tissue to a particular plant. Thus, this method may be useful to determine the levels of cannabinoids across a field and to show levels of overall variability, but is not able to determine how that variability is connected to specific individual plants.

Samples of this type were processed in TIMPL such that while the tissues were still fresh, the leaves and flowers were removed/plucked from the stems and all tissues (flower, leaves, stems) were placed into three respective piles on large mesh screens (Home Depot purchased window screen replacements with frames, extended out to 18 × ~30 inches). One screen was used per field to prevent any chance of cross mixing of samples between fields. The tissues in each pile were spread out and "fluffed" to support efficient air flow around all pieces of the tissues to support efficient drying while also preventing mixing between samples. The screens holding the samples were placed on benches and lab carts in a laboratory room such that air could readily flow around them with a fan gently blowing in the room to circulate the air over the tissue prior to being drawn out of the room via the fume hood (carts holding screens with tissue on them were placed right in front of the fume hood to support efficient air flow). The tissues being dried were kept between 22 °C and 24 °C (71 °F to 74 °F) for several days to ensure complete drying while also keeping the temperature low enough to be confident that no heat conversion of THCA to THC would occur. The relative humidity level in Pullman in the summer is typically lower than 35%, usually in the 20% to 30% range, which is highly conducive to rapid drying of plant tissues. There was no evidence of any molding of samples occurring under these conditions. The samples were all completely dry within 2 – 3 days.

Each of the three tissue piles per field was then separated into two equal sized samples, one to be retained at WSU TIMPL for analysis and the other to be sent to Hops Extract Corporation of America (Hopsteiner) for analysis. So, for each field for each sampling day (30 day preharvest and 15 day preharvest), there were 6 separate samples bagged – 2× with flower tissue, 2× with leaf tissue and 2× with stem tissue. Total of 12 bags for the two days.

Prior to extraction for cannabinoid compositional analysis (potency testing) the leaf:flower samples were mixed at a ratio of 37:30, respectively, by mass, crushed lightly by hand and mixed, then separated into 10 separate samples for each field, with each sample constituted such that it appears to be "representative", i.e., having approximately the same amount of leaf and flower tissue as the other samples. Each separate sample was thus a biological replicate as it contained leaf and flower material that had been slightly crushed to support easy separation into 10 samples, but had not been homogenized and thus turned into one single pooled sample. Each of these 10 samples was then ground to a powder and extracted using the standard approved method provided by the WSDA that utilized methanol as extraction solvent followed by filtration to remove tissue particles and subsequent analysis by HPLC over C18 column with UV detection. Briefly: the samples were ground in a ball mill to a fine powder and a small amount of this powder was placed into a plastic extraction vial with methanol then added to extract the cannabinoids. The samples were allowed to incubate at room temperature for 15 minutes then centrifuged at 20,000 ×g to pellet out all plant powder. The resulting supernatant (methanol solution containing dissolved cannabinoids) was then analyzed by HPLC using UV-based detection, per the standard WSDA method.

In the case of samples analyzed at WSU, the HPLC used was a Waters ACQUITY UPLC with inline PDA detector for UV analysis, with post-PDA analysis by mass spectrometry also performed to provide for extended cannabinoid analysis and to verify identity of all compounds detected and quantified. A standard curve using 12 separate cannabinoids was used in the analysis, with at

least 7 concentrations for each standard compound, to allow for accurate quantitative determination of all compounds, including THC and THCA.

Each sample contained the following tissues at approx. representative ratios for the field at time of collection, based on our observation and previous data collection performed by IHEMPAWA and Trace Analytics Laboratories:

- Flower (30% of mass)
- Young and old leaf (37% of mass)
- Medium stems and leaf stems (20% of mass)
- Old stem/stalk (13% of mass)

For whole plant remediated samples, only the flower and leaf were used for chemical analysis, so the stem mass (33%) was then used to adjust the values obtained for THC and other cannabinoid levels to determine a final, by total plant composition (each final value is divided by 0.67 to obtain the final value determined for the cannabinoid). All values were calculated on a dry weight basis.

Processing and drying of top 1/3 plant samples (2020 method):

The top 1/3 plant samples were analyzed in two ways. One method followed the WSDA IFR 2020 protocol, which generates a "representative sample" for the field. The second method allowed us to evaluate actual variability within the plants across the fields. This was done as outlined below.

Samples were collected in the field into large paper bags as outlined above, with whole branches of plants collected from the top 1/3 of individual plants being placed into a common bag. Two branches per plant were collected so that we had enough to provide samples for the two labs involved in the analysis (TIMPL and Hopsteiner). At least 11 plants were sampled per field (typically 12 – 13) to ensure that enough branches were available with sufficient leaf and flower tissue each for the downstream analysis. This method was deemed useful to determine the levels of cannabinoids across a field and to show levels of overall variability, and was also able to determine how that variability was connected to specific individual plants because the flower/leaf tissues to be evaluated in a single sample came from a single stem. Two types of samples were generated from these collected tissues as outlined below.

Samples of this type were processed in TIMPL such that while the tissues were still fresh, the leaves and flowers were removed/plucked from the stems and all tissues (flower, leaves, stems) were placed into three respective piles on large mesh screens (Home Depot purchased window screen replacements with frames, extended out to 18 × ~30 inches). One screen was used per field to prevent any chance of cross mixing of samples between fields. The tissues in each pile were spread out and "fluffed" to support efficient air flow around all pieces of the tissues to support efficient drying while also preventing mixing between samples. The screens holding the samples were placed on benches and lab carts in a laboratory room such that air could readily flow around them with a fan gently blowing in the room to circulate the air over the tissue prior to being drawn out of the room via the fume hood (carts holding screens with tissue on them were placed right in front of the fume hood to support efficient air flow). The tissues being dried were kept between 22 °C and 24 °C (71 °F to 74 °F) for several days to ensure complete drying

while also keeping the temperature low enough to be confident that no heat conversion of THCA to THC would occur. The relative humidity level in Pullman in the summer is typically lower than 35%, usually in the 20% to 30% range, which is highly conducive to rapid drying of plant tissues. There was no evidence of any molding of samples occurring under these conditions. The samples were all completely dry within 2 – 3 days.

The branches from each field, after being dried, are individually placed into Ziploc bags and labeled such that each plant's tissues would be kept separate from the other plants. A total of 10+ samples were retained at WSU TIMPL for analysis and another 10+ samples per field were sent to Hops Extract Corporation of America (Hopsteiner) for analysis. So, for each field for the 15 day preharvest sampling day, there were 20+ separate samples bagged.

Each individual sample (per plant in each field) was then processed such that the leaves and flowers from each stem were stripped off the stem and combined into a single sample (also containing small "stem pieces" (which were typically the petioles). This sample was then partially crushed to mix the tissue well and divided into two sub-samples per plant. One of these samples was used separate from all other samples for extraction and individual plant cannabinoid profiling as outlined below. The second sample was combined with the similar samples from the other 9 individual samples per field into one large sample, which was then mixed and sub-divided into 10 samples, where each sample was a "whole field representative sample" of the field. Those 10 samples were each crushed completely, ground, and then extracted as outlined below to be used for cannabinoid potency testing. Thus, these were 10 "biological" replicates per field and not technical replicates generated from homogenized tissue or a subsampled single extract.

We thus had 20 samples to analyze per field for the top 1/3 plant collected sample type. Ten of those were used for potency testing per the 2020 IFR method, to compare against the 2019 method, and the other 10 were used for evaluation of plant to plant variability within the fields and overall variability of the method in general.

Prior to extraction for cannabinoid compositional analysis (potency testing) of either sub-sample type for the top 1/3 plant sample type, the total combined leaf and flower material of each individual sub-sample was ground to a powder. Although the IFR called for each sample to be passed through a 1.5×1.5 mm mesh screen to remove any non-powdered stem tissues and then used for the analysis, we did not do so because our ball mill system (TissueLyser) completely powdered all samples to a fine powder with no stem pieces (or any other tissue piece) remaining. In other words, there was no need to sieve a sample that was already a fine powder and thereby either extend significantly the time required for sample processing or increase the risk of sample-to-sample contamination by a sieve. Each of the 10 samples per sample type was then extracted using the standard approved method utilizing methanol as extraction solvent followed by filtration to remove tissue particles and subsequent analysis by HPLC over C18 column with UV detection, as described above.

In the case of samples analyzed at WSU, the HPLC used was a Waters ACQUITY UPLC with inline PDA detector for UV analysis, with post-PDA analysis by mass spectrometry also performed to provide for extended cannabinoid analysis, as described above.