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Steep Hill Study Provides Evidence of Substantial Pesticide Contamination in California Cannabis Clones

Steep Hill

Pesticide use during clone production remains present through the growth cycle of the plant

NEWS PROVIDED BY
Steep Hill →
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BERKELEY, Calif., Sept. 22, 2017 /PRNewswire/ -- **Steep Hill Labs, Inc.,** the global industry leader in cannabis testing and analytics, today released a study, entitled *"**Study of Pesticides in Cannabis Plant Clones**"* (see http://landing.steephill.-com/cleanclones for the full white paper) which shows that – whether through direct use or via cross-contamination – the presence of pesticides in the California cannabis supply is endemic and can be sourced to the persistent presence of pesticides in clones.

In making the announcement, CEO Jmichaele Keller said, "When we released the first study on pesticides in October of 2016, many growers approached **Steep Hill** saying that they did not use pesticides, they were organic, they were trying to do the right thing for their patients and consumers. After hearing this over and over again, we knew there was something wrong in the supply chain. It dawned on me, IT'S IN THE CLONES. We started formulating a plan to discover the root of the problem, because our mission is to make sure that growers have all of the tools and expertise needed to succesfully pass new California regulations. We undertook this study to understand the concern of our clients who were also perplexed by the pesticide reports we were issuing. The conclusions in the attached study indicate a significant number of failures at the clone stage. We realized that serious problems in the California cannabis supply chain could result in 2018 if the very source material from which the cannabis was being grown for large scale production was already contaminated with pesticides failing current regulations. We at **Steep Hill** want to work together with California regulators, growers, product manufacturers and the medical community to solve this problem for the safety and success of the California cannabis supply."

"The report documents clone sources and locations, descriptions of how samples were prepared and analyzed, and the comprehensive findings," Reggie Gaudino, Ph.D and author of the study remarked. "A crucial issue in the industry is that very few growers are breeding to deliver genetic stability, unlike other crops which have been stabilized over decades utilizing sophisticated agricultural processes. Stable genetic lines provide the ability to grow from seed and thus produce hardy, reproducible product. We need to work together to insure that traditional practices in the industry are re-examined and changed in light of this data."

Donald Land, Ph.D, and a co-author of the report remarked, "When the 'mothers' of clones are contaminated with pesticides, particularly those that are systemic, so too are the clone offspring. We look forward to working with the California cannabis industry to establish best practices to substantially reduce the need for the use of pesticides as a whole, and to help set the standard for clean clone production in the future."

Authors of this study include: Anthony Torres, Wilson Linker, Donald Land Ph.D, Reggie Gaudino, Ph.D.

For more information about cannabis testing, please visit the **Steep Hill** website: http://steephill.com

ABOUT STEEP HILL

Steep Hill Labs, Inc. is the world's leading cannabis science and technology company with extensive expertise in lab testing, remote testing, genetics, R&D, and the licensing of our intellectual property to strategic partners across the globe. No other cannabis company brings each of these areas of expertise into one highly synergistic whole. **Steep Hill's** foundation was built on testing and analyzing medical and recreational marijuana to ensure compliance with public safety standards. In 2008, **Steep Hill** opened the first commercial cannabis lab in the United States and the company has been on the cutting edge since its inception. **Steep Hill** is expanding throughout the United States, and globally. With the goal of helping the rest of the world adopt "best practices" in cannabis testing, the company also provides expert consulting services to legislators and regulators in many countries, states, and municipalities around the world.

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Study of Pesticides in Clones

Research Summary

Steep Hill

Authors of this study include: Anthony Torres, Wilson Linker, Donald Land Ph.D, Reggie Gaudino, Ph.D

Premise: With increased testing of cannabis derived samples for pesticides, numerous detections of low-to-moderate levels of pesticide contamination (10's of ppb or less) from a number of growers claiming to use "organic" or "clean green" growing methods prompted us to investigate possible sources. The most prevalent pesticide detected in these cases was Myclobutanil, most commonly used as a treatment for mold infestations. Numerous postings on message boards from growers and clone producers indicate frequent use of Myclobutanil-containing commercially available pesticide products (and others) and a common belief that such use many weeks prior to harvest would not lead to detectable residues in the harvested materials or products refined therefrom. We hypothesize that pesticides used in this manner would persist and, particularly, if the growing medium (soil, rock wool, etc.) were contaminated in the application process, levels of the contaminants in the plants may be present in significant amounts at harvest even without reapplication late in the process.

Study Goal: Determine whether and at what point in the supply chain pesticides are being introduced.

Conclusion: Pesticide use occurs during clone production, and that use remains present through the growth cycle of the plant, and can be tracked through at least 1 generation of passage (e.g., making a clone from the clone; longer term studies in progress). Purchasers of the treated clones likely do not know that they can lead to contamination in the harvested product, even if the grower does not apply pesticides in their practice.

Rationale and Underlying Motivation

Steep Hill Labs has recorded numerous examples of detection of “low” (a few to a few tens of ng/g or ppb) levels of pesticides in cannabis plant material (flowers and leaves) and derived products from growers who were adamant that no such materials were ever applied to the plants or other components of their process. Since this behavior is self-reported, we seek objective means to assess the route of contamination.

Pesticide contamination can occur because growers: (1) knowingly use inputs that contain pesticides (such application may be known to some in the operation, but not others), (2) knowingly use a product whose pesticide content was unknown to them (lack of education, lack of proper labelling), (3) grow in locations that are subject to pesticide “drift” from the application by a neighbor (wind, water, soil, insects, animals).

Direct intentional or unintentional application of pesticides to multiple plants (the typical mode of application when used to control infestation during later stages of the growth cycle) is known to often lead to detection at much higher levels, up to hundreds of ppm - thousands of times higher than the levels observed for the samples in question. While some pesticides are reported to degrade when exposed to ambient conditions and could lead to low level detection, the numerous reports of abstinence from use, often from producers believed to be trustworthy, spurred us to look for further explanations of the phenomenon. Pesticides are commonly detected over a wide range of concentrations (low parts per billion (ppb) to tens or hundreds of parts per million (ppm) - a range that covers 5 to 6 orders of magnitude) confounding the determination.

Thus, during the planning stages, Steep Hill acquired clones with growth media, as well as fresh soil and water samples from the same sources used after transplantation from cooperative clients in this category. We found no evidence of contamination in the bulk soil and water that were used in the later stages of production, however we did detect a range of contamination levels in many of the clones and in the clone growth media. Next, we obtained numerous clones from many commercial sources to carry out this study to estimate the prevalence of contamination of commercially obtained clones.

Materials and Methods

Clone Sources and Locations

Clones were initially sourced by donations from clients, then expanded by soliciting clone donations from the surrounding areas to our Berkeley, California location. However, there were too few donations, so Steep Hill began purchasing clones directly from local clone producers or dispensaries. Clones were sourced primarily from Northern California; however, a small number of samples (17 in total) were obtained from Southern California (Los Angeles, specifically). A total of 124 clones in total were analyzed, some of which were selected to establish a first passage generation for additional studies. It should be noted that the samples were not obtained in a statistically randomized fashion and, for many, the producer of the clones was unknown and could, potentially have originated from fewer common sources.

Sample Preparation and Separation

Fresh leaf material can contain significant amounts of moisture, while our pesticide methods for cannabis plant material were validated for market ready dried material. A standard extraction protocol optimized and validated for fresh leaf tissue analysis and currently used for chemical analysis as part of our Phenosight™ program was used to ensure that sufficient analyte was recovered during extraction to allow accurate detection in the calibration range established on our Shimadzu NeXera X2 LCMS8050 HPLC/triple quadrupole mass spectrometer. Leaf material was extracted in an appropriate volume of acetone and subsequently filtered at 0.2 μm prior to analysis using chromatographic separation using a Phenomenx C18 column and our standard Methanol/Water Formic Acid/Formate mobile phase gradient parameters, as described previously.

Non-leaf material (soil, rock wool, other growing/support media) was prepared using methods typically used for edibles, with the final dilution factor being similar to the dilution used for the fresh leaf material to achieve similar signal response from the triple quadrupole mass spectrometer.

Sample Analysis

Batch data files were analyzed using Shimadzu Lab Solutions software, with both semi-automated and manual integration and verification of analytes using multiple product ions from MRM (multiple reaction monitoring) transitions specific for each analyte. Semi-automated analysis included programming the appropriate MRM's for pesticides, as well as establishing ≥ 5 point calibration curves for each analyte, setting a minimal acceptance level of at least 95% similarity, and validating with calibration challenge and matrix spike samples. A comparison of the California and Oregon action limits for pesticides overlapping between the two states is presented in Figure 1. The pesticides in the figure are presented in the following order: Abamectin-B1a, Abamectin-B1b, Bifenazate, Bifenthrin, Chlormequat, Daminozide, Etoxazole, Fenoxycarb, Imazalil, Imidacloprid, Myclobutanil, Paclobutrazol, Pyrethrins-I, Pyrethrins-II, Spinosad-A, Spinosad-D, Spiromesifen, Spirotetramat, and Trifloxystrobin. Pesticide action limits (OR)/proposed action limits (CA) are shown in parts per million (ppm, μg/g). All chromatographic data was peer reviewed by another member of the research team.

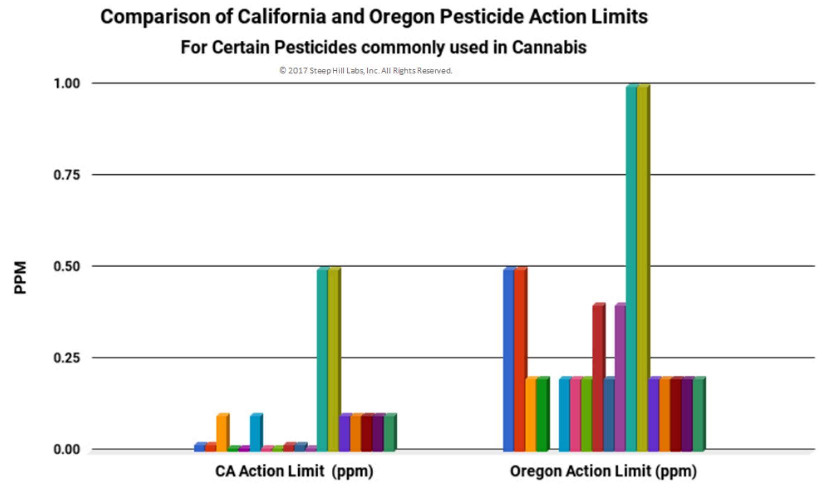

Figure 1: Comparison of certain California and Oregon pesticide action limits

Results and Discussion

Detection Levels

124 clones were sourced for testing. Only 17 (13.7% of the total) showed no detectable pesticides. Of the clones tested, including the clones that had no detectable pesticides, only 22.6% passed the current California Cannabis Regulations for Pesticide Thresholds in market ready cannabis. This is a significant number of failures, at the clone level. This data was the first revelation that there would be serious problems in the California Cannabis supply chain if the very starting material from which the Cannabis was being sourced for large scale production was already contaminated with pesticides that would fail current regulations. See Figures 2 and 3 below.

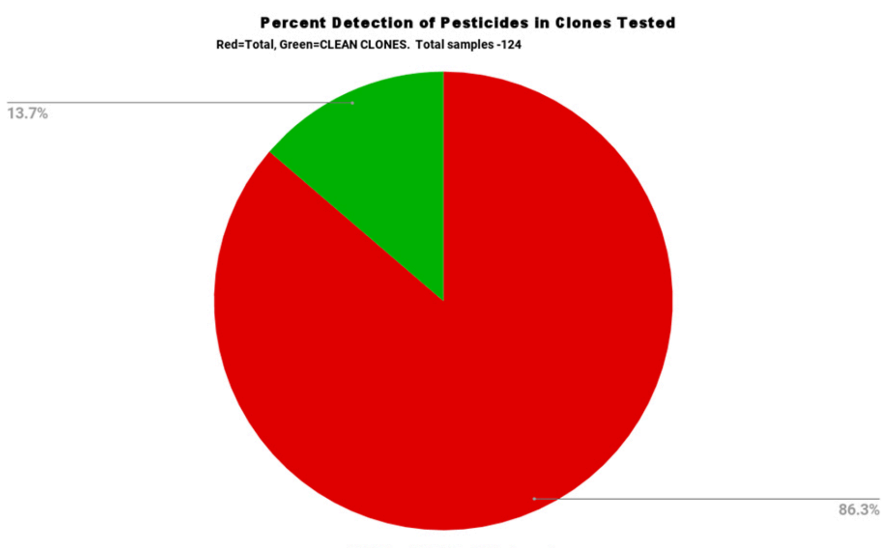

Figure 2. Distribution of clones with or without detectable pesticides; Green represents the proportion of clones tested with no detectable pesticides while red represents the proportion of clones tested with detectable pesticides.

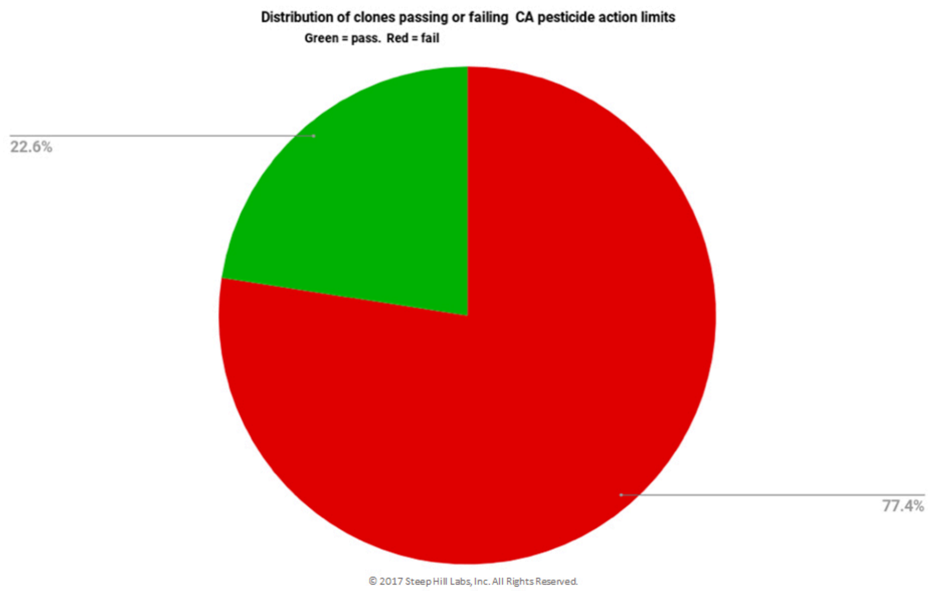

Figure 3: Number of clones tested that passed or failed the proposed California Pesticide thresholds for market ready cannabis. The proportion represented by the red portion of the graph are failures.

The threshold limits include very low levels, some of which could potentially be degraded by environmental conditions, thus potentially rendering some of the products passable by harvest. This led to another revelation/realization, many pesticides are systemic or exist in the soil/growth media, away from many of the very environmental influences that would degrade/inactivate the pesticides and persist far longer than published dissipation rates. Thus, there is not a very high probability that the levels seen in the clones tested would be remediated by any natural/environmental exposure means, and thus there is little likelihood the number of plants that result from these contaminated clones would pass, if they started with significant levels of pesticides.

When we mentioned some of our findings to long time growers known to us, we were informed of "lore" regarding the number of generations required to eliminate pesticides from newly acquired germplasm in the form of clones. Cannabis lore reported to us suggests 4 to 5 generations of clone- mother-clone passage are necessary. It should also be noted that Steep Hill began testing growth support/media (rock wool, polymer, cocoa, peat blocks) as well as the clones, and found that, in many cases, the growth medium was itself, to varying degrees, also contaminated (data not shown). In some cases, the large amount of systemic pesticide (such as myclobutanil) in the growth medium would likely provide a long-term application reservoir if the clone growth medium were transplanted and remained in contact with the root system.

Two questions, "What were the types and levels of pesticides seen in the clones tested?", and "Are any of the clones contaminated to the point that the resulting plants would fail?", are addressed in the following data. Figure 4, below, presents the distribution of pesticides in the clones tested.

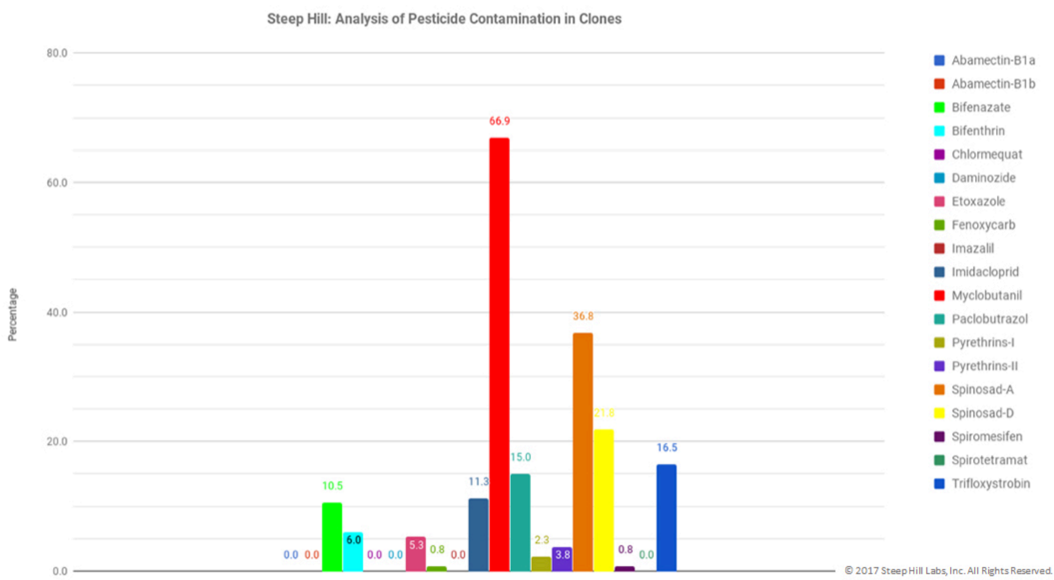

Figure 4: Number of positives for each pesticide type analyzed.

Many of the clones had more than one type of pesticide on them. The most frequently detected pesticide was Myclobutanil, detected in ~67% of all the clones tested. The next most abundant were Spinosad-A and Spinosad-D, detected in 36.8% and 21.8% of the clones tested, respectively. These results are in keeping with the general trend of Myclobutanil being by far the most often detected pesticide in market ready flower and extracts/concentrates.

Figure 5, below, illustrates the total number of each pesticide hit on the left, the number of those pesticide hits that were over the California threshold level in the middle panel, and the % of the total hits that would fail the current California regulations for the 124 clones tested in this study.

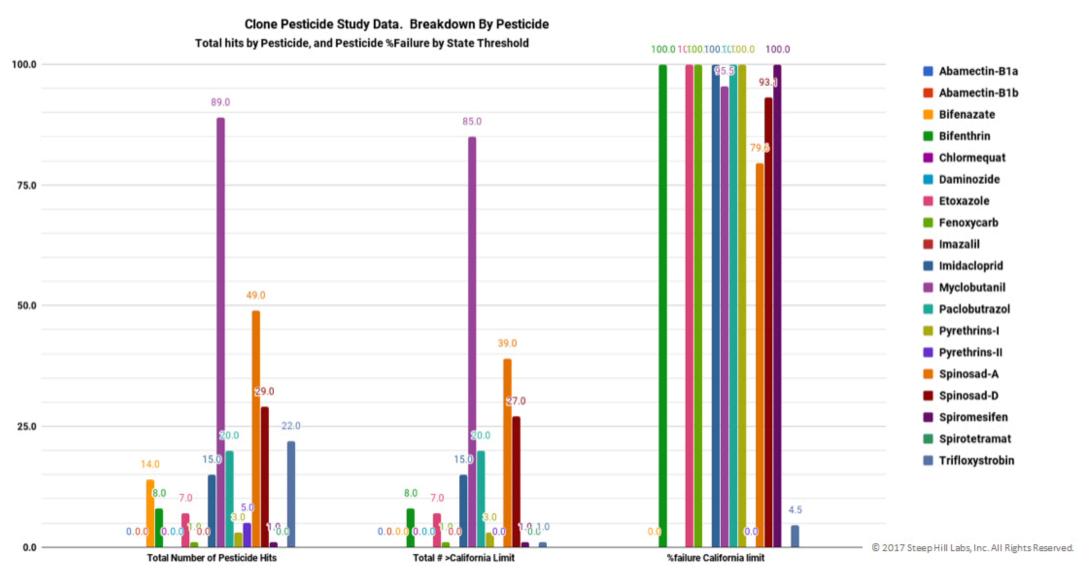

Figure 5: Analysis by Pesticide type, total number of hits, % of hits that fail, and percentage of hits for this study that would fail proposed California regulations.

As visualized in the data above, many, if not all, the pesticide hits resulted in failure for that pesticide. Many of the clones would have failed for two or more pesticides, with each of the pesticides causing failure individually. It should be noted that the level of failure would have been reduced, but in some cases not enough to cause a significant difference in the supply chain, when substituting the Oregon regulations for the California regulations. See Figure 6 below.

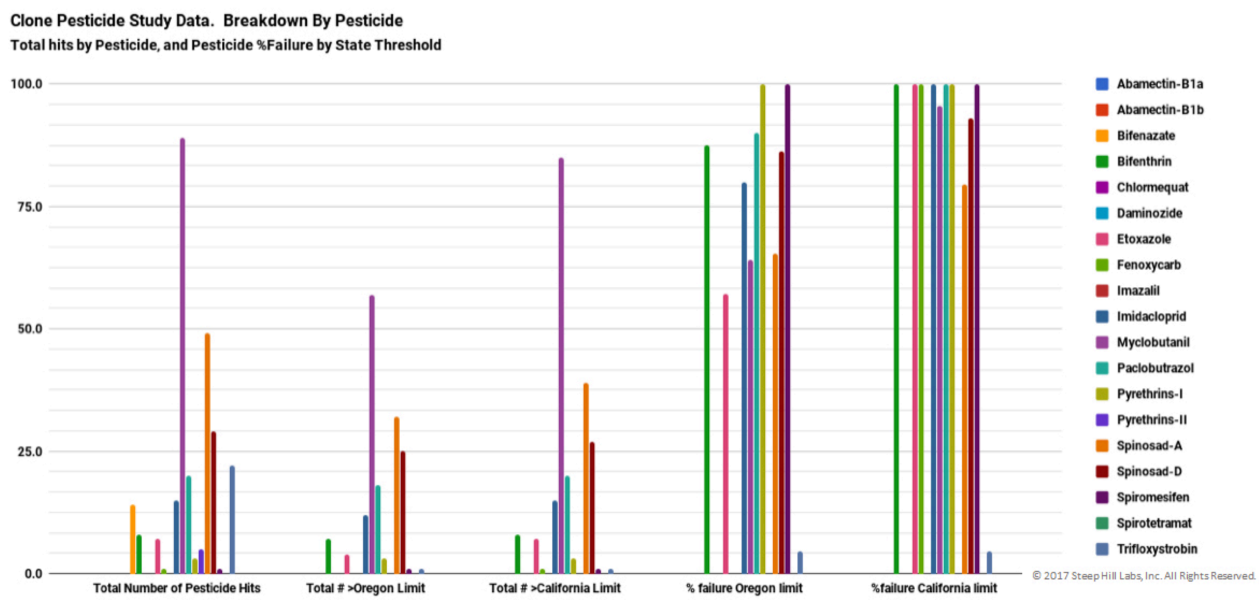

Figure 6: Figure 5 with comparison to Oregon Action limits added.

Given the large number of clones that tested positive for pesticides, the question remained whether the levels seen would be sufficient to find significant amounts in a plant grown to term. It could be argued that a 5ppb detection level in a clone would not leave behind sufficient pesticide residue to be detected months later in a plant that has obviously produced thousands of times more mass. Therefore, we analyzed the levels of detection in terms of the ratio over the current California regulation thresholds. The results are presented in Figure 7 below.

The worst cases are easily distinguished, and the data should be viewed in light of the fact that inside the plant or in the soil, degradation of the pesticides may not be occurring at a rapid pace. It should also be remembered that many growers simply place the clone, growing medium and all, into the next cultivation platform, whether it be soil, or aquaponics, etc. So, if the original growth medium contains significant levels of pesticide, that pesticide can continue to be transported into the growing plant by the original, contaminated support media, now made part of the next phase of cultivation.

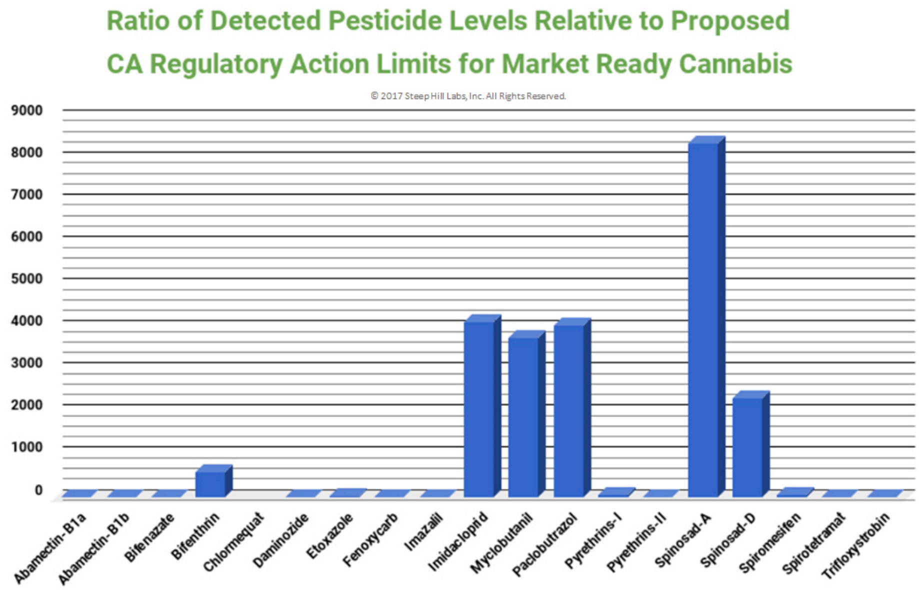

Figure 7: Ratio of detected pesticide levels above action thresholds for the most contaminated clones.

Discussion

Steep Hill started this study wanting to identify where in the supply chain pesticides were becoming a problem, but quickly identified that the problem is endemic: Pesticide use starts from clone propagation in the Cannabis industry.

Close analysis of the industry and the issues associated with large scale cultivation of popular strains reveals why these practices came to be. One of the main issues in the industry is that there has been scarce breeding for stability in the industry, one of the main focuses of other cash crops. Stabile lines provide the ability to grow from seed and thus produce hardy, reproducible product, eliminating the need for the use of clones to get product reproducibility and thus also reduce the use of pesticides needed to ensure high recovery of newly created clones. Cannabis coming from the underground/black market has mostly not had the opportunity for this type of agriculture. Thus, an annual plant is often preserved in its vegetative state for years or even decades, to be the source of future generations of clones with properties similar to the “mother plant.” This is where the first steps towards needing pesticides begins. As the vegetative plant is kept alive, more aggressive methods of keeping the plant healthy need be applied, including pesticides. Many of these pesticides become systemic, and now clones produced from this plant are already dosed with pesticide. However, that is not the end, in order to boost production and thus recovery of clones from already stressed plants, additional pesticides are included in the rooting media used to help establish roots on an already apically differentiated, chlorophyll producing tissue, so that these new plants, also grown vegetatively, can be used as future donors. Thus, in order to support the production of larger numbers of clones, pesticides have become part of the recipe for success. Pesticides eliminate opportunistic invaders both at the microbe and the insect level, helping boost both the indoor and outdoor success of fragile clones.

Testing of the soil/growth media/support showed that the non-plant material also showed detectable levels of pesticide, ranging from lower levels consistent with run off, to higher levels consistent with the support being dipped in the pesticide solution and being used as “sponge” for long term delivery. This is consistent with levels seen in the plants of over 8,300 times the threshold limit in the current California regulations. Again, this is a practice that helps decrease losses and increases the numbers of successful clones for sale. This may be a practice that is likely to increase as the number of new growers without access to seed stock, and the demand for planting ready clones goes up. An extension of this study is still ongoing to determine how many serial passages (making a succession of clones, each from a previous generation) is r equired for the residue to fall below detectable limits, based on different starting levels of application.

Location seemed to not be a factor in the pesticide usage observed, as the majority of clones from both Northern and Southern California were contaminated with pesticides, some at an alarmingly high level. With the exception of the 13.7% of the clones that tested free of any pesticides, many of the clones even failed at the more permissive Oregon threshold levels. Thus, the use of
pesticides seems to be a practice that is in use, regardless of the region in which the clones were sourced, in keeping with a practice that is gaining widespread acceptance and use. A follow up study regarding the prevalence of this practice should be conducted in other states to determine if the practice is common in locations outside of California.

From the data presented herein, less than 14% of 124 randomly selected clones from different regions were free of any pesticide residue, and 77.4% of the clones tested failed current proposed California Cannabis pesticide regulations, it is clear there is a need for clone monitoring. Clone monitoring will help eliminate this source of pesticide contamination in the California Cannabis supply chain. Given these findings, it is likely that a large proportion of the California Cannabis
supply currently being readied for harvest will face challenges with respect to pesticide detection. Of particular concern are the extract/concentrate product types, since the starting material may contain a significant amount of pesticide contamination, and the extraction process serves to further concentrate the compounds in question.

In Summary

Less than 14% of 124 randomly selected clones from different regions were free of any pesticide residue, and 77.4% of the clones tested failed current proposed California Cannabis pesticide regulations, it is clear there is a need for clone monitoring. Clone monitoring will help eliminate this source of pesticide contamination in the California Cannabis supply chain. Given these findings, it is likely that a large proportion of the California Cannabis supply currently being readied for harvest will face challenges with respect to pesticide detection. Of particular concern are the extract/concentrate product types, since the starting material may contain a significant amount of pesticide contamination, and the extraction process serves to further concentrate the compounds in question.