BOTEC
Analysis Corporation

Testing *Cannabis* for Contaminants

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BACK OF THE ENVELOPE CALCULATIONS

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I. Introduction

With the passage of Initiative 502, the State of Washington is in a unique position to chart the development and regulatory oversight of a new agricultural industry, the production of *Cannabis* for recreational use. This paper will attempt to address *Cannabis* quality from the perspective of potential contaminants in the final product. Like any agricultural commodity, *Cannabis* may be attacked by pests or pathogens and require treatment with insecticides, acaricides, fungicides, and potentially other crop protection agents (CPAs). The crop may be grown in soils contaminated by organic chemicals from earlier agricultural operations, exposed to spray drift from adjacent fields, or even take up toxic metals from the soil. Many of these types of potential contamination are known in other crops, where tolerance levels are established through health risk analysis. Residue presence is monitored to assure the safety of vegetable and meat products and beverages. The one other smokable commodity, tobacco, stands apart from the regulatory strategies that address food monitoring, and we will attempt to summarize the current situation with regard to contamination detection for that product; we will examine the regulatory environment that addresses tolerances for contaminants in tobacco and other crops to propose policies that will support sustainable and safe *Cannabis* agriculture.

In the United States, the Environmental Protection Agency (EPA) regulates the agricultural use of biocide products in order to protect human health and the physical environment under the auspices of the Federal Food, Drug and Cosmetic Act (FFDCA) and its amendments, while the Food and Drug Administration (FDA) enforces EPA tolerances in foods, and the United States Department of Agriculture (USDA) is the responsible enforcement entity for pesticide tolerances in meats, poultry, and eggs, and under narrow circumstances, tobacco products (EPA 2013a). The EPA Office of Pesticide Programs accomplishes these tasks by evaluating pesticide toxicology, evaluating biocide behavior under field conditions, registering compounds for use on a crop-by-crop basis, establishing field usage patterns, and establishing limits for the chemical residues allowed on harvested products. Due to the illegality of *Cannabis*, however, the EPA has not been in a position to approve any agricultural products or practices for *Cannabis* cultivation. Moreover, agronomic and pest management research in support of *Cannabis* production were effectively halted in this country well before both the post-WWII boom in chemical pest control and the subsequent emergence of the integrated pest management (IPM) concept in the 1970s (Huffaker et al. 1980). This has left both policymakers and well-intentioned producers with very few guidelines to follow, in terms of reducing the harms to the public environment, protecting the health of the consumer, and allowing for the sustainable production of high-quality *Cannabis*.

In lieu of approved inputs for *Cannabis*, this paper will at times analyze regulations and present residue tolerances for comparable crops, such as leafy vegetables, teas, and spices. On one hand, any food product is comparable to *Cannabis*: both are intended for oral consumption, with or without cooking, so analogies for residue tolerances would seem to be straightforward. On the other hand, consumption via smoking or vaporizing is significantly different from consumption via eating. When a consumer smokes *Cannabis*, he exposes the product to extreme temperatures and combustion, which can cause chemical transformations that might not have occurred if the product were prepared for oral consumption. In fact, it has been shown that many hazardous compounds tobacco smoke

are also emitted in *Cannabis* smoke, including ammonia, cyanide, heavy metals, and polycyclic aromatic hydrocarbons, although the relative amounts of these deleterious materials differed significantly between the two types of smoke (Moir et al. 2008; it should be noted that this study did not address pyrolysis of applied materials). It has recently been demonstrated that *Cannabis* smoke may contain significant amounts of pesticide residues when present on the product (Sullivan et al. 2013). Moreover, products that are smoked rather than eaten take a different path inside the human body, being absorbed by the lungs, and bypassing the stomach and subsequent "first pass" metabolism by the liver, prior to distribution in the bloodstream. For these reasons, this paper takes regulations for tobacco as the closest comparable proxy for smoked *Cannabis*.

It is our expectation that eventually the EPA will examine the use of pesticides in *Cannabis* production. Certainly, there are strong arguments for doing so. *Cannabis* is a high-value and popular crop, consumed by millions of Americans every year. Each of these people is potentially at risk for exposure to harmful chemicals in an unregulated production environment. Secondly, the historically illegal and underground production of *Cannabis* suggests additional cause to suspect the chemical purity of *Cannabis*. On the other hand, if pesticide choices and use guidelines can be established, it is likely that health risk to consumers, workers, and the environment can be significantly reduced. In any case, until the EPA establishes approved inputs for *Cannabis*, other preventative measures ought to be taken by the state.

The LCB is encouraged to engage with the Washington State Department of Agriculture (WSDA) early in the process of regulating *Cannabis* production. Two Divisions within the WSDA regulate pesticide use: the Pesticide Management Division and the Food Safety Division. These offices would determine registration requirements, exemptions from registration, "state only" registrations, and experimental use permits. Note that adjuvants added to active pesticide ingredients (e.g.: surfactants, drift control agents, stickers) are also regulated, and permits for their use are also issued by the WSDA (Johansen 2012).

This paper will identify compounds that should be monitored and address analytical methods for monitoring their residues. We reviewed over 700 technical papers describing analytical methods that have been applied to the compounds from the initial survey, and will summarize those findings.

II. Pesticides in Tobacco

Tobacco, like *Cannabis*, is principally intended for consumption via combustion and inhalation. Unlike *Cannabis*, the agricultural inputs used with tobacco have been rigorously studied and regulated (although pesticide residue monitoring is far from uniform, as we will see). For this reason, regulations for tobacco productions are an especially useful comparison for similar regulations for *Cannabis*.

Tobacco is produced by a large and powerful agricultural industry that makes significant use of pesticides to protect the growing crop and harvested tobacco during curing, manufacturing, and storage. As many as 16 separate applications of pesticides are recommended by tobacco companies just in the interval between greenhouse seed sowing and transplantation into the field (Taylor 1994). In the United States, however, regulation of pesticide use in tobacco presents unusual dichotomies when compared to pesticide regulation in food production. As described above, the EPA is charged with regulating pesticide use in agriculture, and for literally hundreds of agricultural commodities there are explicit maximum residue level (MRL) tolerances that may not be exceeded. By the early 1990s, there were at least 37 pesticides approved by the EPA for use on tobacco crops in this country, although since that time many of these materials have had their registrations for use in tobacco cancelled (Anon. 2003).

While EPA approvals address requirements for worker protective gear and health monitoring, application rates and frequencies, pre-harvest intervals, and other factors, the EPA has determined that pesticide residues in finished tobacco pose a negligible incremental risk to health when compared to the direct effects of nicotine and other combustion products in tobacco smoke. The EPA has chosen to not regulate pesticide residues in domestically grown tobacco, and it does not issue residue level guidelines for tobacco products at this time. This is despite the stipulation in the Family Smoking Prevention and Tobacco Control Act (Public Law 111-31, H.R. 1256, June 22, 2009, which provided the FDA with authority to protect public health by regulating tobacco products) that "Beginning 2 years after the date of enactment of the Family Smoking Prevention and Tobacco Control Act, a tobacco product manufacturer shall not use tobacco, including foreign grown tobacco, that contains a pesticide chemical residue that is at a level greater than is specified by any tolerance applicable under Federal law to domestically grown tobacco." At the point in 2011 when this law would seem to have been enforceable, the FDA issued a statement to the tobacco industry that included the following statement: "To determine whether there are pesticide residue tolerance levels applicable to domestic tobacco, the Food and Drug Administration (FDA) consulted with the U.S. Department of Agriculture (USDA) and U.S. Environmental Protection Agency (EPA). According to USDA and EPA, under their laws there are currently no established tolerance limits for pesticide chemical residues that apply to domestically grown tobacco. If such a tolerance is established, we plan to provide this information to tobacco product manufacturers" (Deyton 2011). At this writing, the situation has not changed, and in this country pesticide

monitoring has been largely left to the discretion of the industry, with few exceptions, described below. The U.S. tobacco industry is known to have vigorously lobbied against stricter pesticide controls and public disclosure of residue levels (McDaniel et al. 2005).

The situation in the European Union is similar. Tobacco industry organizations have been formed to promulgate Good Agricultural Practices (GAP) and pesticide Guidance Residue Levels (GRLs), in part to deflect governmental regulation across the EU. The Paris based Centre de Coopération pour les Recherches Scientifiques Relatives au Tabac (CORESTA) is one such organization with membership largely drawn from the tobacco industry, which publishes guideline manuals that address numerous aspects of tobacco agricultural practices (Anon. 2005), GRLs (Anon. 2013), technical aspects of residue analysis (Anon. 2008), and guidance for sampling the tobacco production supply chain (Anon. 2012). A table of GRLs from the CORESTA 2013 Guidelines series is reproduced in Appendix 1 of this document, and lists suggested residue levels for 120 CPAs. A number of the compounds listed (DDT, Endrin, etc.) have been banned for use in the U.S. and the EU for decades, but may still contribute residue signatures, owing to their extremely long environmental half-lives.

The expressed goal of CORESTA is the promotion of science-based approaches to tobacco production practices and the maintenance of sustainable tobacco agriculture across the EU, yet their organization has also received criticism for concealing sources of funding for experts provided to governmental panels addressing pesticide concerns, lobbying for raising tolerance limits for materials of questionable safety, and general lack of transparency (McDaniel et al. 2005). These tactics mirror industry efforts to create confusion around the science of second-hand tobacco smoke in this country (Ong & Glantz 2001).

The EPA regulates which pesticides can be applied during production and subsequent manufacturing, but in general it has not regulated pesticide residue levels in the final products of domestic producers (Stephenson 2003; Deyton 2011). Instead, the agency requires evaluation of residue behavior in tobacco from field trials, and has demanded additional data when pesticides or known harmful breakdown products exceed 0.1 parts per million (ppm) in the harvested or cured crop (Stephenson 2003). Additional information regarding pyrolysis products (compounds formed during combustion) in tobacco smoke have been requested when residue levels have exceeded this threshold, but empirically determined levels in smoke have not been determined to warrant further regulatory action by the agency.

Unlike the market for *Cannabis*, the American tobacco market consists of both domestic and imported product. Since imported tobacco is not subject to EPA regulations in the production stage, scrutiny is applied upon import instead. For imported tobacco and the portion of domestic tobacco that the federal government procures under the tobacco price support program, the USDA monitors the residues of 20 pesticides that are otherwise prohibited for tobacco use in the U.S. This monitoring regime protects domestic growers from unfair competition from foreign producers and mitigates the public's exposure risk to highly toxic pesticides banned for use in this country. Since there are currently no means for legal import of *Cannabis*, this aspect of consumer protection need not be emulated in *Cannabis* policy.

Later sections will discuss compounds that were found in the survey and Steep Hill Lab's own database to be in common use with California medical *Cannabis* growers. Many of these compounds are in use and have been strictly regulated for other crops. While the accepted testing for such a different crop as a tomato may seem inappropriate (and unvalidated) for *Cannabis*, it can inform us on the toxicity of the compound. Using existing residue tolerance standards for those compounds on food and tobacco as a guide, this section will identify candidate substances for monitoring and regulation.

III. Contaminants of Interest

A. Pesticide residues

In order to direct our attention to the range of chemicals likely to be found in the *Cannabis* regulated by Initiative 502, we conducted a survey of pesticides used by growers providing for the California medical *Cannabis* industry. To protect their crops, growers of medical *Cannabis* in California mainly turn to over-the-counter insecticides, acaricides, and fungicides (note that there has been no formal registration of any pesticides for *Cannabis* cultivation in California). These compounds are listed in Table 1, along with their manufacturers, trade names, pest targets, and registration status with the EPA. Where available, manufacturer websites, academic, and government sources were used to collect Material Safety Data Sheets (MSDSs) and general toxicological parameters for these compounds, and are given in Table 2. Several of these materials are common horticultural products or food additives, which were, by prevailing expert opinion, exempted from tolerance regulation under the FFDCA and its amendments. These include essential oil preparations, insecticidal soaps, and mineral products (silica dusts, sulfur, etc.). This latter group may include compounds that fell into the FDA Food Additives Amendment of 1958 "generally regarded as safe" (GRAS) category for food additives, which included over 700 materials either deemed safe through expert consensus or by their lengthy use in the food industry. These unregulated materials are considered toxicologically and environmentally benign.

Table 1. Pesticides in the Medical Cannabis Industry in California: Initial Survey

Product Name	Primary Active Ingredient	EPA Pesticide Type	Manufacturer	Product Type	CAS#	EPA PC Code	Insects	Mites	Fungi	Bacteria	OMRI	EPA Status
Zero Tolerance Pesticide	"Essential Oils" (mixed)	Antimicrobial, biochemical, conventional chemical	Quick Trading Company	pesticide	57-06-7	004901	20047	Y	N	N	Y	Registration review
Orthene	acephate	Conventional chemical	Whitmire Microgen	pesticide	30560-19-1	103301	Y	Y	N	N	N	Registration review
Shuttle O	acequinocyl	Conventional chemical	OHP	pesticide	57960-19-7	006329	N	Y	N	N	N	Registered
Avid	avermectin (B1?)	Antimicrobial, conventional chemical	Syngenta	pesticide	71751-41-2	122804	Y	Y	N	N	N	Registration review
Neem Oil	azadirachtin	Biochemical, conventional chemical	Dyna-Grow	pesticide	108168-76-9	121701	Y	Y	N	N	N	Registration review
K Plus Neem	azadirachtin	Biochemical, conventional chemical	Organica	pesticide	108168-76-10	121701	Y	Y	N	N	N	Registration review
Azamax	azadiractin	Biochemical, conventional chemical	General Hydroponics	pesticide	108168-76-11	121701	Y	Y	N	N	Y	Registration review
Azatrol	azadiractin	Biochemical, conventional chemical	Gordons	pesticide	108168-76-12	121701	Y	Y	N	N	Y	Registration review
Einstein Oil	azadiractin	Biochemical, conventional chemical		pesticide	108168-76-13	121701	Y	N	N	N	N	Registration review
Azasol	azadiractin	Biochemical, conventional chemical	Arborjet	pesticide	108168-76-14	121701	Y	N	N	N	N	Registration review
Serenade	*Bacillus subtilis*	Biochemical, conventional chemical	Agraquest	biofungicide	-	006480	N	N	Y	Y	Y	Registration review
Serenade Max	*Bacillus subtilis*	Biochemical, conventional chemical	Agraquest	biofungicide	-	006480	N	N	Y	Y	Y	Registration review
Silica Blast	*Bacillus subtilis*	Biochemical, conventional chemical	Botanicare	biofungicide	-	006480	N	N	Y	N	N	Registration review
Safer Caterpillar Killer	*Bacillus thuringiensis*	Biochemical, conventional chemical	Woodstream	biopesticide	68038-71-1	006400	Y	N	N	N	N	Reregistration
Floramite	bifenazate	Conventional chemical	Uniroyal Chemical	pesticide	149877-41-8	000586	N	Y	N	N	N	Registration review
Hot Pepper Wax	capsaicin	Biochemical, conventional chemical	Hot Pepper Wax	repellent	404-86-4	070701	N	N	N	N	N	Registration review
Zero Tolerance Fungicide	cinnamon oil	Biochemical	Quick Trading Company	fungicide	-	129066	N	N	Y	N	N	None
SNS 203	clove oil	-	Sierra Natural Science	pesticide	-	-	Y	N	N	N	N	None
Earth Tone Garden Fungicide	copper octanoate	Conventional chemical	Espoma	fungicide	20543-04-8	023306	N	N	Y	N	N	Registered
Mildew Cure	cottonseed oil	Conventional chemical	Safergro	fungicide	8001-29-4	031602	N	N	Y	N	Y	None
Orange Guard	d-limonene	Antimicrobial, biochemical, conventional chemical		repellent	138-86-3	079701	N	N	N	N	N	Registration review
Phosphoload	daminozide	Conventional chemical		pgr	1596-84-5	035101						Registration review
Topload	daminozide	Conventional chemical		pgr	1596-84-6	35102	N	N	N	N	N	Registration review
Flower Dragon	daminozide	Conventional chemical		pgr	1596-84-7	35103	N	N	N	N	N	Registration review
Insect Dust	diatomaceous earth	Conventional chemical	St Gabriels Organics	pesticide	7631-86-9	072605	Y	N	N	N	Y	Registration review
Spectracide Immunox	diazinon	Biochemical, conventional chemical	Spectrum Group	pesticide	333-41-5	057801	Y	Y	Y	N	N	Registration review

Table 1. Pesticides in the Medical Cannabis Industry in California: Initial Survey cont'd…

Product Name	Primary Active Ingredient	EPA Pesticide Type	Manufacturer	Product Type	CAS#	EPA PC Code	Insects	Mites	Fungi	Bacteria	OMRI	EPA Status
Basudin	diazinon	Biochemical, conventional chemical	Ciba	pesticide	333-41-6	57802	Y	Y	Y	N	N	Registration review
Physan 20	dimethyl benzyl ammonium chloride	Antimicrobial, conventional chemical	Maril Products Inc.	fungicide	53516-76-0	069104	N	N	Y	Y	N	Reregistration
Etherel	ethephon	Conventional chemical	Bayer	pgr	16672-87-0	099801	N	N	N	N	N	Registration review
Zeal	etoxazole	Conventional chemical	Valent	pesticide	153233-91-1	107091	Y	Y	N	N	N	Registered
Preclude TR	fenoxycarb	Biochemical, conventional chemical	Whitmire Microgen	pesticide	72490-01-8	125301	Y	Y	N	N	N	Registration review
Garlic Barrier	garlic extract (Oil)	Biochemical, conventional chemical	Garlic Research Labs	repellent	8000-78-0	128827	N	N	N	N	Y	Registration review
No Powdery Mildew	geranium oil	Biochemical, conventional chemical	Greenway Nutrients	fungicide	8000-46-2	597500	N	N	Y	N	N	None
No Spider Mites	geranium oil	Biochemical, conventional chemical	Greenway Nutrients	pesticide	8000-46-3	597500	N	Y	N	N	N	None
Oxidate	hydrogen dioxide	Antimicrobial, biochemical, conventional chemical	Biosafe Systems LLC	fungicide	7722-84-1	000595	N	N	Y	Y	N	Registration review
Hydrox	hydrogen peroxide	Antimicrobial, biochemical, conventional chemical	Green Planet	fungicide	7722-84-2	000595	N	N	Y	Y	N	Registration review
Fungaflor TR	imazalil	Conventional chemical	Whitmire Microgen	fungicide	35554-44-0	111901	N	N	Y	N	N	Reregistration
Advantage	imidacloprid	Antimicrobial, conventional chemical	Bayer	neonicotinoid pesticide	138261-41-3	129099	Y	Y	N	N	N	Registration review
Merit	imidacloprid	Antimicrobial, conventional chemical	Bayer	neonicotinoid pesticide	138261-41-3	129099	Y	Y	N	N	N	Registration review
Liquid Ladybug	lemon grass oil	Antimicrobial, biochemical, conventional chemical	ASAP Products LLC	pesticide	8007-02-1	040502	N	Y	N	N	N	Registration review
Eagle 20	myclobutanil	n/s	Dow Agrosciences	fungicide	88671-89-0	128858	N	N	Y	N	N	None
Gognats	not specified	?	Hydrodyamics International	pesticide	-	-	Y	Y	N	N	N	?
Bushmaster	paclobutrazol	Conventional chemical		triazole pgr	76738-62-0	125601	N	N	N	N	N	Registration review
Gravity	paclobutrazol	Conventional chemical		pgr	76738-62-1	125601	N	N	N	N	N	Registration review
Pure Spray Green	petroleum oil	Antimicrobial, conventional chemical	Petro Canada	pesticide	8008-20-6	063501	Y	Y	Y	N	Y	None
OrganocideTM Plant Doctor	phosphoric acid	Antimicrobial, conventional chemical	Organic Laboratories Inc.	fungicide	7664-38-2	076001	N	N	Y	N	N	Registration review
Green Cure	potassium bicarbonate	Biochemical, conventional chemical	H And I Agritech	fungicide	298-14-6	073508	N	N	Y	N	N	None
Insect Killing Soap	potassium salts of fatty acids	Antimicrobial, biochemical, conventional chemical	Safer Brand	pesticide	67701-09-1	079021	Y	N	N	N	Y	Registration review
Total Release Fogger	propoxur butoxide	Biochemical, conventional chemical	Doktor Doom	pesticide	114-26-1	047802	Y		N	N	N	Registration review
Xclude	pyrethrin	Antimicrobial, biochemical, conventional chemical	BASF	pesticide	8003-34-7	069001	Y	Y	N	N	N	Registration review

Table 1. Pesticides in the Medical Cannabis Industry in California: Initial Survey cont'd…

Product Name	Primary Active Ingredient	EPA Pesticide Type	Manufacturer	Product Type	CAS#	EPA PC Code	Insects	Mites	Fungi	Bacteria	OMRI	EPA Status
Spider Mite Knockout	pyrethrin	Antimicrobial, biochemical, conventional chemical	Doktor Doom	pesticide	8003-34-7	069001	Y	Y	N	N	N	Registration review
Pro Control Plus	pyrethrin	Antimicrobial, biochemical, conventional chemical	Whitmire Microgen	pesticide	8003-34-7	69001	Y	Y	N	N	N	Registration review
Garden Insect Spray	pyrethrin	Antimicrobial, biochemical, conventional chemical	Bonide Products	pesticide	8003-34-7	69001	Y	Y	N	N	N	Registration review
Earth Tone Insect Control	pyrethrin	Antimicrobial, biochemical, conventional chemical	Espoma	pesticide	8003-34-7	69001	Y	Y	N	N	N	Registration review
Don't Bug Me	pyrethrins	Antimicrobial, biochemical, conventional chemical	Foxfarm	pesticide	8003-34-7	69001	Y	Y	N	N	N	Registration review
Spider Mite Control	rosemary oil	Biochemical, conventional chemical	Sierra Natural Science	repellent	8000-25-7	597700	N	N	N	N	N	None
SNS 217 Organicide	rosemary oil sesame oil	Biochemical, conventional chemical Conventional chemical	SIerra Natural Science Organic Laboratories Inc.	pesticide pesticide	8000-25-7 8008-74-0	597700 072401	N Y	Y Y	N Y	N N	N Y	None None
Oleotrol M	soybean oil	Biochemical, conventional chemical	Natural Forces LLC	fungicide	8001-22-7	031605	N	N	Y	N	Y	Registration review
Fungus Pharm	soybean oil	Biochemical, conventional chemical	Pharm Solutions	fungicide	8001-22-7	031605	N	N	Y	N	N	Registration review
Monterey Garden Insect Spray	spinosyn	Biochemical, conventional chemical	Lawn and Garden Products Inc	pesticide	131929-60-7	110003	Y	N	N	N	Y	Registration review
Captain Jacks Dead Bug Brew	spinosyn	Biochemical, conventional chemical	Bonide Products	pesticide	131929-60-7	110003	Y	Y	N	N	N	Registration review
Forbid 4F	spiromesifen	Conventional chemical	Bayer	pesticide	283594-90-1	024875	Y	Y	N	N	N	Pending registration
Actinovate	streptomyces lydicus	Biochemical	Natural Forces LLC	biofungicide	-	006327	N	N	Y	N	N	Registered
Sucrashield	sucrose octonoate esters	Biochemical, conventional chemical	Natural Forces LLC	pesticide	42922-74-7	035300	Y	Y	N	N	Y	Registered
Garden Fungicide	sulfur	Antimicrobial, conventional chemical	Safer Brand	fungicide	7704-34-9	077501	N	N	Y	N	Y	Registration review
Bonide Sulfur	sulfur	Antimicrobial, conventional chemical	Bonide Products	fungicide	7704-34-9	077501	N	N	Y	N	N	Registration review
Earth Tone 3N1	sulfur	Antimicrobial, conventional chemical	Espoma	pesticide	7704-34-9	077501	Y	Y	Y	N	N	Registration review
SNS 244	thyme oil (thyme camphor)	hort. oils	Sierra Natural Science	fungicide	89-83-8	080402	N	N	Y	N	N	Registration review
Earth Tone Insecticidal Soap	vegetable oil (sulfonated?)	Antimicrobial, conventional chemical	Espoma	pesticide	61790-19-0	079013	Y	Y	N	N	N	None
Protek	carbendazim	Antimicrobial, conventional chemical	?	cleaner	10605-21-7	128872	N	N	N	N	N	None
Bang	-	?	?	pesticide	-	-	Y	Y	N	N	N	?
SM90	-	?	Nutrilife	?	-	-						?
Nuke Em	-	?	FS Plant Products	pesticide	-	-	Y	Y	Y	N	N	?
Dr Nodes	-	?	?	pgr	-	-	N	N	N	N	N	?
Dr Do Right	-	?	?	pesticide	-	-	Y	N	N	N	N	?
PM Wash	-	?	NPK Industries	fungicide	-	-	N	N	Y	N	N	?
Mighty Wash	-	?	NPK Industries	cleaner	-	-	N	N	N	N	N	?
Power Wash	-	?	NPK Industries	cleaner	-	-	Y	N	N	N	N	?

Table 2. Pesticides in the Medical Cannabis Industry in California: MSDS and toxicology data.

Product Name	Primary Active Ingredient	Form	MSDS pdf	LD50 Oral mg/kg	LD50 Dermal mg/kg	LD50 Inhalation mg/l	Carcinogen?	Source
Zero Tolerance Pesticide	"Essential Oils" (Mixed)	liquid	http://z-tolerance.com/uploads/msds/MSDS_ZT_PESTICIDE_ReadyToUse_2011.pdf	?	?	?	n/a	-
Orthene	acephate	liquid	http://www.americanpest.net/docs/msds/orthene-msds.pdf	500-5000	2000	2-20	Possible	npic.orst.edu
Shuttle O	acequinocyl	liquid	http://greenhouse.ucdavis.edu/pest/pmsds/Shuttle%20O.pdf	>5000	>2000	>0.84	Not Likely	cdpr.ca.gov
Avid	avermectin (B1?)	liquid	http://www.cdms.net/LDat/mp770018.pdf	10	330->200	3.5	Weak	pmep.cce.cornell.edu
Neem Oil	azadirachtin	liquid	http://www.dyna-gro.com/Website%20pdf%20Files/MSDS%20Neem%20Oil.pdf	3540->5000	>2000	>2.4	n/a	"
K Plus Neem	azadirachtin	liquid	http://www.kellysolutions.com/erenewals/documentsubmit/KellyData%5CID%5Cpesticide%5CMSDS%5C70191%5C70191-1%5C70191-1_ORGANICA_K+NEEM_INSECTICIDE_FUNGICIDE_12_30_2005_1_37_15_PM.pdf	3540->5000	>2000	>2.4	n/a	"
Azamax	azadiractin	liquid	http://generalhydroponics.com/site/gh/docs/prod_msds/azamax.pdf	3540->5000	>2000	>2.4	n/a	"
Azatrol	azadiractin	liquid	http://www.pbigordon.com/pdfs/Azatrol-MSDS.pdf	3540->5000	>2000	>2.4	n/a	"
Einstein Oil	azadiractin	liquid	http://www.hydrofarm.com/downloads/fc/msds%20001_32382.jpg	3540->5000	>2000	>2.4	n/a	"
Azasol	azadiractin		http://www.arborjet.com/msds/AzaSolMSDS.pdf	3540->5000	>2000	>2.4	n/a	"
Serenade	*Bacillus subtilis*		http://www.agraquest.com/docs/labels-msds/SerSoil-MSDS-062110.pdf (Serenade Soil)	>5000	>5000	>1.4	n/a	agraquest.com
Serenade Max	*Bacillus subtilis*	liquid	http://www.agraquest.com/docs/labels-msds/SerMax-MSDS-051811.pdf	> 2,000	> 2,000	> 0.63	Not Likely	agraquest.com
Silica Blast	*Bacillus subtilis*	liquid	http://sunlightsupply.s3.amazonaws.com/documents/product/732485_MSDS.pdf	> 2,000	> 2,000	> 0.63	Not Likely	agraquest.com
Safer Caterpillar Killer	*Bacillus thuringiensis*	liquid	http://www.saferbrand.com/resource/MSDS/EN/5160.pdf	>5000	>5000	>2.0	Not, per OSHA	saferbrand.com
Floramite	bifenazate	liquid	http://greenhouse.ucdavis.edu/pest/pmsds/Floramite.PDF	>5000	>5000	>5.2	Not Likely	greenhouse.ucdavis.edu
Hot Pepper Wax	capsaicin	liquid	http://www.gemplers.com/docs/msds/7253.pdf	>2500	n/a	n/a		Mouse
Zero Tolerance Fungicide	cinnamon oil	liquid	http://z-tolerance.com/uploads/msds/MSDS_ZT_FUNGICIDE_ReadyToUse_2011.pdf	?	?	?	n/a	z-tolerance.com
SNS 203	clove oil	liquid	http://sierranaturalscience.com/wp-content/uploads/2012/02/sns203-MSDS1.pdf	5000	n/a	n/a	n/a	sierranaturalscience.com
Earth Tone Garden Fungicide	copper octanoate	liquid	http://espoma.com/p_consumer/pdf/labels/GardenFungicide_MSDS.pdf	>2000	>2000	n/a	Not, per OSHA,IARC	espoma.com
Mildew Cure	cottonseed oil	liquid	http://1000bulbs.com/pdf/mildew-cure-msds.pdf	>90 mL/kg	n/a	n/a	Not Likely	fscimage.fishersci.com
Orange Guard	d-limonene	liquid	http://1000bulbs.com/pdf/orange-guard-msds.pdf	4400	>5000	n/a	Not Likely	sciencelab.com
Phosphoload	daminozide		http://www.hydrofarm.com/downloads/fc/DM%20Phosphoload%20MSDS_21227.pdf	8400	>1600	>147	Probable (EPA)	pmep.cce.cornell.edu
Topload	daminozide		x	8400	>1601	>148	Probable (EPA)	pmep.cce.cornell.edu
Flower Dragon	daminozide		x	8400	>1602	>149	Probable (EPA)	pmep.cce.cornell.edu
Insect Dust	diatomaceous earth	solid	http://www.domyownpestcontrol.com/msds/Insect_Dust_msds.pdf	n/a	n/a	n/a	Proven (human - IARC)	sciencelab.com
Spectracide Immunox	diazinon	aerosol	http://www.homedepot.com/catalog/pdfImages/a3/a30ac883-2eaf-4486-af3c-9eb589b91e58.pdf	300-400	3600	3.5	Not Likely	extoxnet.orst.edu
Basudin	diazinon	liquid	could only find this "Basudin" http://www.basudin.com/bindex.jsp, emailed them, choice btwn Basudin 600 EW, 600 EC or 10 GR	300 - 400	3600	3.5	Not Likely	extoxnet.orst.edu
Physan 20	dimethyl benzyl ammonium chloride	liquid	http://www.physan.com/Resources/MSDS-Physan%2020.pdf	240	n/a	n/a	n/a	sciencelab.com
Etherel	ethephon	liquid	http://www.bayercropscience.com.au/resources/uploads/msds/file9067.pdf	3400 - 4229	139->5000	> 5	No	pmep.cce.cornell.edu
Zeal	etoxazole	liquid	http://www.valent.com/Data/Labels/0268rev3.pdf	> 5000	> 5000	> 2.28	Not Likely	toxnet.nlm.nih.gov
Preclude TR	fenoxycarb		http://florawww.eeb.uconn.edu/msds/preclude_TR_msds.pdf	> 1600	> 5000	> 480	n/a	pmep.cce.cornell.edu
Garlic Barrier	garlic extract (Oil)	liquid	http://www.planetnatural.com/wp-content/uploads/garlic-barrier-msds.pdf	850 (mouse)	n/a	n/a	n/a	sciencelab.com
No Powdery Mildew	geranium oil	liquid	http://sunlightsupply.s3.amazonaws.com/documents/product/739155_MSDS.pdf	5000	n/a	n/a	Not listed: ACGIH,IARC	sunlightsupply.s3.amazonaws.com
No Spider Mites	geranium oil	liquid	http://sunlightsupply.s3.amazonaws.com/documents/product/704765_MSDS.pdf	5000	n/a	n/a	Not listed: ACGIH,IARC	sunlightsupply.s3.amazonaws.com
Oxidate	hydrogen dioxide	liquid	http://bwgs.blob.core.windows.net/docs/OxiDateRTUMSDS.pdf	5000	> 2000	n/a	7540 mg/kg/day (mouse)	toxnet.nlm.nih.gov
Hydrox	hydrogen peroxide	liquid	In folder	5000	> 2000	n/a	7540 mg/kg/day (mouse)	toxnet.nlm.nih.gov
Fungaflor TR	imazalil	solid	http://betterplants.basf.us/products/msds-and-labels/fungaflor_msds.pdf	227 - 343	4200 - 4880		n/a	extoxnet.orst.edu
Advantage	imidacloprid	liquid	http://www.westernu.edu/bin/safety/msds/VET-MED-VACS/MSDS%20Advantage%20Bayer.pdf	450	>5000	>69 (aerosol), >5323 (dust)	Not	extoxnet.orst.edu

Table 2. Pesticides in the Medical Cannabis Industry in California: MSDS and toxicology data, cont'd.

Product Name	Primary Active Ingredient	Form	MSDS pdf	LD50 Oral mg/kg	LD50 Dermal mg/kg	LD50 Inhalation mg/l	Carcinogen ?	Source
Merit	imidacloprid	solid	http://www.cdms.net/LDat/mp8H8001.pdf (0.3)	450	>5000	>69 (aerosol), >5323 (dust)	Not Likely	extoxnet.orst.edu
Liquid Ladybug	lemon grass oil	liquid	http://www.arbico-organics.com/downloads/liquid-ladybug-msds.pdf	>5000	n/a	n/a	Not Likely	57aromas.com
Eagle 20	myclobutanil		http://www.precisiondallas.com/Images/msds/Eagle%2020EW.pdf	1750 - 1800	n/a	n/a	Not Likely	precisiondallas.com , toxipedia.com
Gognats	not specified	liquid	http://sunlightsupply.s3.amazonaws.com/documents/product/720327_MSDS.pdf	?	?	?	?	sunlightsupply.s3.amazonaws.com
Bushmaster	paclobutrazol		http://www.sunlightsupply.com/docs/Emerald%20Triangle/BushMaster%20MSDS.pdf	5346	> 1000	369	n/a	cdms.net, epa.gov
Gravity	paclobutrazol		http://www.sunlightsupply.com/docs/Emerald%20Triangle/Gravity%20MSDS.pdf	5346	> 1001	369	n/a	cdms.net, epa.gov
Pure Spray Green	petroleum oil	liquid	http://www.treecarescience.com/uploads/Labels/Insecticides/Pure%20Spray%20Green/Pure%20Spray%20Green%20MSDS.pdf	>5000	>2000	>2500	Not listed: ACGIH,IARC	treecarescience.com
OrganocideTM Plant Doctor	phosphoric acid	liquid	http://www.homedepot.com/catalog/pdfImages/0d/0d3a51ec-0ac6-4fe7-b65b-e3516baaef0f.pdf	1530	2740	n/a	n/a	sciencelab.com
Green Cure	potassium bicarbonate	solid	http://www.planetnatural.com/wp-content/uploads/green-cure-msds.pdf	2700	>5000	>2.3	n/a	planetnatural.com
Insect Killing Soap	potassium salts of fatty acids	liquid	http://www.ces.ncsu.edu/fletcher/programs/xmas/pesticides/labels/Safer-insect-killing-soap-rtu-msds.pdf	74000	>5000	>2	Not listed: ACGIH,IARC	ces.ncsu.edu
Total Release Fogger	propoxur butoxide	aerosol	http://sunlightsupply.s3.amazonaws.com/documents/product/704400_MSDS.pdf	83 - 150	500	n/a	No	pmep.cce.cornell.edu
Xclude	pyrethrin	liquid	http://www.cdms.net/LDat/mp9II002.pdf	200 - >2600	>2000	> 6000	No	cdms.net, extoxnet.orst.edu, pmep.cce.cornell.edu
Spider Mite Knockout	pyrethrin	aerosol	http://www.kellysolutions.com/erenewals/documentsubmit/KellyData%5COK%5Cpesticide%5CMSDS%5C72804%5C2724-568-72804%5C2724-568-72804_Doktor_Doom_Spider_Mite_Knock_Out_Insecticide_Plant_Spray_F_Tomatoes_and_Vegetables_1_10_2011_3_38_16_PM.pdf	200 - >2600	>2000	> 6000	No	cdms.net, extoxnet.orst.edu, pmep.cce.cornell.edu
Pro Control Plus	pyrethrin	aerosol	http://www.batzner.com/docs/MSDS-Labels/Pro-ControlPlusMSDS.pdf	200 - >2600	>2000	> 6000	No	cdms.net, extoxnet.orst.edu, pmep.cce.cornell.edu
Garden Insect Spray	pyrethrin	liquid	http://www.bonide.com/lbonide/msds/msds857.pdf	200 - >2600	>2000	> 6000	No	cdms.net, extoxnet.orst.edu, pmep.cce.cornell.edu
Earth Tone Insect Control	pyrethrin	liquid	http://espoma.com/p_dealers/PDF/ETInsectRTU-MSDS.pdf	200 - >2600	>2000	> 6000	No	cdms.net, extoxnet.orst.edu, pmep.cce.cornell.edu
Don't Bug Me	pyrethrins	liquid	http://www.planetnatural.com/wp-content/uploads/dont-bug-me-msds.pdf	200 - >2600	>2000	> 6000	No	cdms.net, extoxnet.orst.edu, pmep.cce.cornell.edu
Spider Mite Control	rosemary oil	liquid	http://sierranaturalscience.com/wp-content/uploads/2012/02/sns_217-mite-spray.pdf	5000	n/a	n/a	Not Likely	sierranaturalscience.com
SNS 217 Organicide	rosemary oil sesame oil	liquid liquid	http://sierranaturalscience.com/wp-content/uploads/2012/02/sns_217-mite-spray.pdf http://www.organiclabs.com/Images/MSDS/Plant%20Doctor%20MSDS.pdf	5000	n/a	n/a	Not Likely	sierranaturalscience.com
Oleotrol M	soybean oil	liquid	http://bwgs.blob.core.windows.net/docs/OleotrolMMSDS.pdf	5000	4000	n/a	Not Likely	bwgs.blob.core.windows.net
Fungus Pharm	soybean oil	liquid	http://www.justorganics.net.au/justorganics/Assets/FUNGUS_PHARM_MSDS.pdf	5000	4000	n/a	Not Likely	bwgs.blob.core.windows.net
Monterey Garden Insect Spray	spinosyn	liquid	http://www.biconet.com/botanicals/infosheets/MonteregySprayMSDS.pdf	> 2000	> 5000	> 5.18	No	ec.europa.eu
Captain Jacks Dead Bug Brew	spinosyn	liquid	http://www.bonide.com/lbonide/msds/msds250.pdf (RTU)	> 2000	> 5000	> 5.18	No	ec.europa.eu
Forbid 4F	spiromesifen	liquid	http://pdf.tirmsdev.com/Web/692/15018/692_15018_MSDS_English_.pdf?download=true	> 2000	> 2000	1.8	No	pdf.tirmsdev.com
Actinovate	streptomyces lydicus	solid	http://plantprodmany.compassites.com/?m=product_pdf&u=plantmany&p=products&id=6020001 (SP)	> 5050	> 5050	> 5050	No	plantprodmany.compassites.com
Sucrashield	sucrose octonoate esters	liquid	http://www.naturalforcesllc.com/PDFs/20071129%20NaturalForce%20SucraShield%20MSDS.pdf	10080 - >20000	n/a	n/a	Not Likely	gpo.gov
Garden Fungicide	sulfur	liquid	http://www.saferbrand.com/resource/MSDS/EN/5456.pdf	>5000	>5000	>5	Not Likely	cospl.coalliance.org
Bonide Sulfur	sulfur	solid	http://www.bonide.com/lbonide/msds/msds141.pdf				Not Likely	cospl.coalliance.org
Earth Tone 3N1	sulfur	liquid	http://espoma.com/p_dealers/PDF/ET3n1RTU_MSDS.pdf	>5000	>5000	n/a	Not Likely	cospl.coalliance.org
SNS 244	thyme oil (thyme camphor)	liquid	http://sierranaturalscience.com/wp-content/uploads/2012/02/sns244-RTU-MSDS-Final.pdf	2840	> 5000	n/a	n/a	spectrumchemical.com
Earth Tone Insecticidal Soap	vegetable oil (sulfonated?)	liquid	http://espoma.com/p_dealers/PDF/ETSoapRTU-MSDS.pdf	>5000	>5000	n/a	n/a	espoma.com
Protek	carbendazim	liquid	http://www.protekchemical.com/sites/default/files/Pro	> 15000	> 2000	n/a	n/a	superway.com

Some, but not all of the pesticides encountered in the California survey are formally registered by the EPA for use in other crops; in these cases, the EPA has established maximum residue levels (MRLs) for those commodities (EPA 2012); these tolerance levels are presented in Table 3. These may serve as useful starting points for establishing residue tolerance guidelines or limits for *Cannabis*. A table of current guideline residue levels (GRLs) promulgated by CORESTA for use by the European tobacco industry is presented in Appendix 1, and includes many compounds that have been banned from use in this country for many decades. The position of CORESTA is that laboratories testing tobacco products should have the capability of identifying even these old compounds, which may persist in agricultural lands today.

Table 3. Selected pesticide tolerance levels for food and feed commodities. All values are in ppm; ranges reflect values among subgroups.[1]

Primary Active Ingredient	Lettuce	Spinach	Spearmint Peppermint	Berry	Cherry	Straw-berry	Vine fruit	Wheat grain	Hops	Nuts	Dry Herbs
acephate	10		27								
acequinocyl				0.5	0.5		1.6		4	0.02	
avermectin	0.1		0.01			0.02			0.2	0.01	0.03
azadirachtin											
bifenazate			25	15	5	1.5	1		15	0.2	
daminozide											
diazinon	0.7		0.7		0.2	0.5	0.75			0.5	
ethephon				20-30	10			2		0.5-0.8	
etoxazole			10	0.5						0.01	
fenoxycarb											
imazalil								0.1			
imidacloprid	3.5	3.5		0.5-3.5		0.5		0.05	6	0.05	48
myclobutanil		0.03	3	20-30	5			0.03	10		
paclobutrazol											
propoxur											
pyrethrins				1	1			3		0.02-1	
spinosyn		8	3.5	0.01-0.7		1			22		22
spiromesifen											
carbendazim											

[1] From: Anon. (2012) Index to pesticide chemical names, Part 180 Tolerance information, and food and feed commodities (by commodity). U.S. Environmental Protection Agency, Office of Pesticide Programs.

Note that one of the compounds from our initial survey (diazinon) is no longer permitted for use in tobacco in the US, and further, that none of the compounds on the USDA residue target list for tobacco appeared on our survey list. Also note that a very few European governments have established residue limits on tobacco for the pesticides encountered in our survey; those threshold values are listed in Table 4. Ethephon is a plant growth regulator that is quickly converted by plants to ethylene gas, which is a ripening hormone in many crops, and thought to promote development of female *Cannabis* plants; its toxicity is regarded as very low. Imidacloprid is a neonicotinoid nerve agent with high selectivity for insect nerve systems, and at present may be the most widely applied insecticide, worldwide. Concerns have recently arisen that Imidacloprid may contribute to honey bee colony collapse disorder (Whitehorn et al. 2012), and the European Food Safety Authority has proposed significant restriction of this compound, along with clothianidin and thiamethoxam (also neonicotinoid insecticides) in the EU, starting on December 1,

2013, to further evaluate effects on bees. This leaves the EU with very few formally approved pesticides for tobacco with specific residue limits.

Table 4. Residue limits for pesticides encountered in the initial survey, for tobacco products in European countries. Data from Stephenson (2003)

	Residue limits (in ppm)		
Pesticide	**Germany[a]**	**Italy[b]**	**Spain[c]**
acephate	[d]	1.5	[d]
diazinon	1.0	[d]	0.02
ethephon	[d]	16.0 (green) 80.0 (cured)	[d]
imadacloprid	[d]	10.0 (green) 50.0 (cured)	5.0

[a] Residue limit on finished products

[b] Limit on green tobacco unless otherwise noted

[c] Limit on dried tobacco

[d] Limits not established for this compound

Pesticide use in *Cannabis* production remains of concern for several reasons. While residues on the marketed product are important metrics for quality, it may be difficult to associate trace residues with human health effects, or these correlations may take years of careful medical research to become detect. Perhaps more importantly, the creation of rational guidelines for pesticide use can serve to protect workers in the production system and the environment. As a high value crop, *Cannabis* will no doubt prompt some growers to use any and all measures to maximize yields, regardless of burdens or risks placed on employees, customers, or their surroundings. This should be prevented by appropriate registrations, inspection, and residue analysis.

B. Microbial Contamination

Cannabis, along with tobacco and most other crops, is subject to contamination by pathogenic fungi, bacteria, plant viruses, protozoa, and other microbial life forms. Many of these may be innocuous symbionts living on the plant without causing damage to the host plant or to consumers, but others can cause serious plant disease or harm consumers. McPartland et al. (2000) subdivided microbial contaminants of *Cannabis* into Group 1 organisms (present on the growing plant in the field, and carrying over into curing and storage) and Group 2, which only infest dead plants, and are saprophytes found on stored products. Each group can cause its own losses and potential risks to health. Characteristics of some of these organisms are summarized in Table 5.

Microbial testing will prove to be an essential component of health and safety testing for *Cannabis* regulated by Initiative 502. *Cannabis* is vulnerable to fungi and bacterial diseases; its growing conditions are often ideal environments for microbial

growth, and even when human disease is not a threat, allergic responses, off flavors, and physical degradation of product are real concerns. Some of the fungi associated with *Cannabis* are also known to infect tobacco (Lucas 1975; McPartland et al. 2000; Bailey 2013). Fungi can produce mycotoxins, some of which are extremely dangerous; those produced by *Aspergillis* and *Fusarium* infecting *Cannabis* have been shown to cause human illness in certain cases (Kurup et al. 1983; Llewellyn 1977).

Table 5. Some microorganisms associated with cannabis (adapted from McPartland et al. 2000).

Organism	Group	Associated Plant or Human Disease
Botrytis cinerea	1	Grey mould
Sclerotinia sclerotiorum	1	Hemp canker
Alternaria alternata	1	Brown blight
Cladosporium herbarum	1	*Cladosporium* stem canker
Epicoccum nigrum	1	Black dot disease (aka: Black spot)
Stachybotrys lobulata	1	Associate with fiber hemp "retting"
Stemphyllium botryosum	1	*Stemphyllium* leaf and stem spot
Fusarium spp.	1	Fusarium stem canker, Foot and Root Rot, toxic metabolites (trichothecenes)
Mucor spp.	1	Not pathogenic, but common on plants
Various bacteria	1	May contribute to desireable curing processes
Aspergillus spp.	2	"Mouldy marijuana"
Aspergillus fumigatus	2	Bronchopulmonary aspergillosis, pneumonitis, alflatoxins
Penicillium spp.	2	Opportunistic infections, off flavors
Mucor spp.	2	Common, not associated with human disease
Rhizopus spp.	2	Opportunistic infections, off flavors

There are currently no accepted standards for safe levels of fungi or bacteria in *Cannabis.* In the absence of standards for *Cannabis,* this paper will examine standards for other products meant for human consumption, such as tobacco and nutritional supplements. For these commodities, standards have been issued by the US Pharmacopeial Convention (USP) and enforced by the FDA. Standards exist on two levels. There are standards for the total mold or fungal content of a product ("Colony forming units" or CFUs, a measure of the total number of viable cells in a sample), and for levels of particular types of mold. Some benign forms are acceptable if below given thresholds, while the more dangerous varieties are treated with zero tolerance.

There exist standardized methods for microbial testing appropriate for *Cannabis*, including viable cell density and more rigorous identifications. Methods for determining numerical levels of molds and bacteria have been established for over 40 years by organizations such as the USP and the National Science Foundation (NSF). Further identification of pathogens utilizes modern techniques such as microscopic inspection, PCR (polymerase chain reaction, preceding DNA analysis), ELISA (enzyme linked immunoassay) and chromatography of microbial toxins.

Molds that have been known to grow on and infect tobacco plants include *Cladosporium, Penicillium, Alternaria, Aspergillus* and *Mucor* (Lucas 1975; Bailey 2013). All of these have also been found to infect *Cannabis* plants (Kurup 1983). Other molds found to infect *Cannabis* plants include *Scopulariopsis, Rhizopus, Fusarium,.* Bacteria associated with *Cannabis* include *E.coli*, *Salmonella,* and *Listeria* (Farr 1989; McPartland 1992; McPartland et al. 2000; Taylor 1982). Dahiya & Jain (1977) tested the direct effects of cannabinoids on 18 species of fungi, and found that THC and CBD inhibited growth of all except *Aspergillus*

niger and *Penicillium chrysogenum*, so particular attention should be paid to these species in stored *Cannabis* products.

Mycotoxins have been shown to cause illness apart from opportunistic infection (Schwartz 1985). Mycotoxins of the greatest concern include aflatoxins produced by *Aspergillus* species (Llewellyn 1977), including aflatoxin B1 (a known hepatocarcinogen), ochratoxins (carcinogens produced by *Aspergillus* and *Penicillium)*, and fumonisin produced by *Fusarium*. Deoxynivalenol and T-2 are toxic trichothecenes produced by *Fusarium*.

Allergic bronchopulmonary aspergillosis is an illness that causes fever and asthmatic symptoms and has been linked to aflatoxins produced by *Aspergillus* species (Llamas 1978; Chusid 1975), particularly *A. fumigatus* but also *A. niger* and *A. flavus*. Growth of *Aspergillus* is common in plants that are not properly dried and cured (Kagen 1981). Aflatoxins have also been known to cause sinusitis in marijuana smokers (Schwartz 1987), a condition also been linked to non-*Aspergillus* species (Schwartz 1992). *Penicillium* species, although used to produce antibiotics, vitamins, and "blue" cheese, have been associated with opportunistic infections in humans, as have *Rhizopus* species (McPartland et al. 2000). *Fusarium* species, along with being virulent plant pathogens, also produce toxic metabolites. *F. graminearum* produces zearalenone, which cause flu-like symptoms, along with trichothecenes such as "T-2 toxins" which produce haemorragic symptoms, and were implicated as biological warfare agents during the Viet Nam era (Rippon 1988). The American Herbal Products Association (AHPA 2013) has proposed the following maximum quantitative limits for aflatoxins in dried, unprocessed herb products:

- Total aflatoxins ($B_1 + B_2 + G_1 + G_2$): 20 µg/kg (ppb)
- Aflatoxin B_1: 5 µg/kg (ppb)

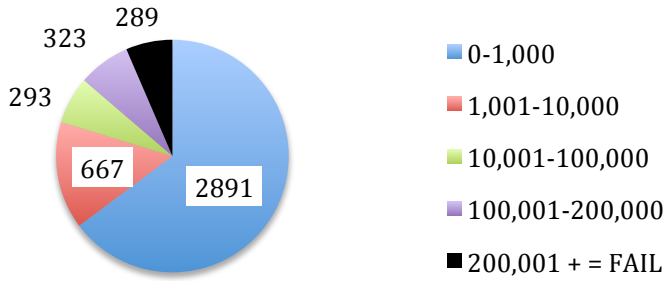
The bacteria *Salmonella muenchen* was found to infect *Cannabis* plants and has been linked to cases of salmonellosis (Taylor 1982) with symptoms including diarrhea, vomiting, fever, and enteritis. Severely immune-compromised individuals such as AIDS patients have been shown to develop mycoses in advanced stages of the disease (Bossche & Mackenzie 1990; Wheat 1995); it is important to set standards to ensure that *Cannabis* consumed by these patients is as free of microbial contamination as possible.

The most common preventable causes of microbial infection of a plant are inadequate attention to conditions that predispose the crop to infection and disease development. These include improper planting density or irrigation practices, inappropriate crop rotation practices, lack of field sanitation such as destruction of crop residues, disinfection of tools, boots or greenhouse benches and equipment, the use of animal or human feces as manure, contaminated water, and poor worker hygiene during harvesting and processing of the plant product (USP 2023). Sound agricultural practices established for many other field crops need to be systematically introduced into *Cannabis* production to reduce the risk of disease outbreaks and microbial residues in cured products.

Regulations and standards for dietary supplements created by the USP, NSF, World Health Organization (WHO), and European Pharmacopeia (EP) have determined what microbial levels present in a product deem it unsafe for human consumption. These

standards are not all in agreement. The NSF tolerates much higher CFU levels than does WHO: 100,000 cfu/g (NSF 2008) compared to only 100 CFU/g (WHO 2012). Steep Hill Laboratories has adopted a grading system based on USP, NSF, and WHO standards; the "Fail" threshold used in this system reflects the value used by the American Herbal Products Association (AHPA) of 200,000 CFU/g; Figure 1 shows the distribution of samples analyzed in 2012 using our adapted grading system, for Total Mold and Yeast CFU.

Figure 1. Total mold and yeast CFU counts in *Cannabis* samples processed at Steep Hil Laboratories, 2012. Data from 4,436 samples.



C. Heavy Metals

Cannabis contamination by heavy metals can be of concern because these elements are not broken down metabolically, instead accumulating in the body, and can cause a variety of health problems, including neurological disorders. Heavy metals are emitted naturally by geological phenomena including volcanic eruptions and erosion, but are also by-products of industrialization and power generation. Metals such as mercury, cadmium, chromium are widely dispersed in the environment, and contaminate water supplies and agricultural soils to varying degrees. The FDA monitors foods for metals contamination and issues manuals that describe numerous methodologies for metals analysis (FDA 2013a). We can consider two broad types of contamination sources: Type 1 sources include uptake from soils, atmospheric deposition, water sources, and fertilizers; Type 2 sources include intentional contamination of *Cannabis* for enhanced profits. Both types of contamination have been reported in the literature.

Cannabis has been reported to hyperaccumulate metals from contaminated soils, and has even been reportedly used to extract cadmium and copper from contaminated soils, with the metals recovered by acid leaching after harvest (Kozlowski 1995). *Cannabis* fertilized with inorganic fertilizers may accumulate trace metals, and even radionuclides, as in the case of tobacco accumulation of polonium-210 (Muggli et al. 2008). Internal tobacco documents showed that cured product contained from 0.33 to 0.36 picocuries of ^{210}Po per gram of plant matter (Ferguson 1997) and could contribute to risks of lung cancer.

Type 2 contamination, though rare, has been reported. In 2008 nearly 150 people in Leipzig, Germany were poisoned by *Cannabis* adulterated with powdered lead metal. The

contamination was apparently performed to increase the market weight (and profit margin) of the product, and resulted in an initial 16 patients admitting themselves to hospitals with severe headaches, insomnia, neuropathies, and "Burton's Lines," greyish bands at the gum line, characteristic of acute lead exposure (Busse et al. 2008). Once law enforcement officials were notified, lead was discovered in hemp supplies, at up to 10% by weight. Some victims had lead levels well above the danger threshold of 80 µg/deciliter of blood; the highest reported was 457 µg/dl, nearly six times the danger level. Glass particles have also been detected in *Cannabis*, presumably with the same intent (Cole et al. 2010). Hopefully, in a regulated marketplace this sort of malicious adulteration will be exceedingly rare.

The American Herbal Products Association (AHPA 2013) has proposed the following levels as maximum quantitative limits for orally consumed herbal products:

- Arsenic 10.0 µg/day
- Cadmium 4.1 µg/day
- Lead 6.0 µg/day
- Mercury[1] 2.0 µg/day

[1]As methyl mercury

D. Pests and other foreign matter

Insects, metal fragments, and other debris are found in food as well as tobacco products, and will likely also be detected in *Cannabis*, particularly as manufactured products enter the marketplace. The FDA considers debris of this kind in food to pose a negligible health hazard, but clearly quality and the user experience is compromised, and has manuals of methods for monitoring (FDA 2013b), which should be consulted to compare standards for different food commodities.

The tobacco industry recognizes that foreign matter (Non-Tobacco Related Matter, NTRM) degrades product quality, but addresses this problem with cultivation, harvesting, curing, and transportation guidelines for producers and manufacturers (Anon. 2010). CORESTA offers similar guidelines to the tobacco industry in Europe (Anon. 2005). At this time, Steep Hill Labs is not monitoring for this type of contamination, since the vast majority of samples submitted are trimmed flower buds or processed products, such as food items and concentrates; we will be considering implementation of this class of monitoring as the industry matures.

PART TWO – METHODS FOR CONTAMINANT DETECTION

IV. Methods for Pesticide Analysis

Any practical analytical method for pesticide residues must be able to isolate, separate, identify, and quantify a large number of compounds at very low concentrations. This has prompted extensive development of "multiresidue" methods, which attempt to measure up to a few hundred target compounds in a single analytical run. Analytical methods must demonstrate selectivity and sensitivity. These terms resemble the statistical concepts of sensitivity (proportion of actual "positives" correctly identified as such) and specificity (proportion of "negatives" correctly identified as such), but in analytical chemistry these terms are defined somewhat differently. Selectivity is the ability to measure individual analytes in complex mixtures without interference by sample constituents or other residues (Vessman et al. 2001). Sensitivity is defined as the ability of a method to measure compounds at very low levels with good precision. The lower limits of detection of a method are statistically defined, and must be relevant to residue tolerance levels. A successful method will still have good linearity and precision at sample concentrations well below a GRL or MDL. Pesticides levels decline after application in the field, from photooxidation, volatilization, and biological degradation, so residues on harvested products are often in the low part-per-million (ppm, μg/g) to part-per-billion range (ppb, ng/g). Analytical methods must also be efficient to develop and maintain, be readily calibrated, and be amenable to automated data processing, storage, and reporting. We will address some of these issues below.

A. Sample Preparation Methods

All analytical methods for organic pesticides start with a representative sample, one that is randomly selected on the basis of the sample type, subdivided, and well mixed. Samples could include expanded leaves in the field, flowers nearing maturity, crop during curing or at any stage of transportation or storage. If relevant, samples may be taken during food preparation or preparation of refined smoking products.

Residual pesticide compounds must be extracted and isolated from the bulk sample matrix and interfering materials. Broadly speaking, the lower the concentration of the target, the more stringent these extraction and cleanup processes must be in order to reliably identify and measure trace residues. The general steps required for pesticide analysis include:

- Sample comminuting (grinding, etc.), mixing to assure representative subsamples
- Extraction with suitable solvents (for selective removal of targets while minimizing extraneous interferences)
- Cleanup of solvent extracts to remove interferences
- Separation of sample components (chromatography)
- Detection and identification of targets (chromatographic detectors, MS, etc.)

Analysis is complicated by the broad range of physical and chemical properties of the target compounds, and the sample matrices from which they must be removed. Pesticides may be acidic, basic, or neutral. They may have widely varying polarity and solubility properties. Many are thermally sensitive, and some are known to bind onto surfaces during isolation.

Sample matrices include liquids (surface waters, groundwater, beverages), soils and sediments, fresh vegetation, fruits and vegetables, and edible cooked products. In the case of *Cannabis*, samples are predominantly in the form of harvested, cured flowers. Separating pesticides and other analytes from the other parts of *Cannabis* plant matter is complex and difficult. The active cannabinoids are produced heavily in glandular trichomes on the leaf and flower surfaces, that are extremely resinous. Many pesticides are hydrophobic; they adhere to or dissolve into these resinous structures and are often difficult to remove. Flower buds may be in the form of refined products (powdered glandular trichomes, or Kief, hashish, tinctures, extracted resins) and cooked products that may also have physical properties that complicate pesticide isolation.

We have found no published research that specifically addressed the requirements for dealing with these matrices in *Cannabis* pesticide residue analysis, and methods that address these issues will have to be developed prior to adoption of analytical methods acceptable to the Board. In the absence of specific research on *Cannabis* matrices, appropriate techniques for *Cannabis* can be gleaned from methods used for other crops (focusing on plant products in dry leaf form, such as tobacco, teas and spices, and environmental media such as soils).

A signature development in sample preparation for pesticide analysis was the use of organic solvents to extract un-dried samples in the presence of salts to aid the separation of aqueous and organic phases. This seemingly simple approach greatly aided further isolation of targets from matrix solids (Luke et al. 1975). This technique was modified and refined extensively, and gave rise to the QuEChERS method, or Quick, Easy, Cheap, Effective, Rugged, and Safe sample preparation. The method has two overall stages: sample extraction, and dispersive solid phase extraction (SPE) cleanup.

During extraction, acetonitrile (ACN) is added to homogenized, ground sample in a disposable centrifuge tube (to avoid losses of volatile pesticides, grinding is often performed in the extraction tube with added dry ice). The tube is shaken thoroughly, a mixture of magnesium sulfate ($MgSO_4$) and sodium chloride (NaCl) is added, and the sample is again shaken vigorously. The salt addition helps break any emulsion that might have formed between residual moisture in the sample and the ACN, and allows separation of and organic phase. Internal standard is added (triphenyl phosphate, TPP, is recommended for general pesticide analysis, although others might be substituted), the sample is shaken, then centrifuged for 5 minutes at 5,000 rpm.

Dispersive SPE cleanup that removes polar interferences follows: a 1 mL aliquot of the organic layer from the first step is added to a 2 mL centrifuge tube, pre-filled with 50 mg of a mixed primary and secondary amine-coated granular sorbent (PSA), and 1 g NaCl, and thoroughly shaken. It is centrifuged again for 5 minutes, and an 0.5 mL aliquot is pipetted into a sample vial for analysis. The resulting sample can be analyzed by various means, but in many commercial analytical laboratories, chromatographic systems (Gas

chromatography, GC, High-pressure Liquid Chromatography, HPLC, etc.) are used to further separate, detect, and identify sample components. These analytical methods are discussed below.

A general workflow for the QuEChERS method is shown in Figures 2 and 3. Agilent, Supelco, Restek, and other major vendors of analytical supplies all now sell pre-packaged QuEChERS kits with minor modifications for different specific methods or matrices, greatly aiding the analyst in choice of materials and specific techniques for a given problem. Analyte recovery with these techniques are excellent and reproducible, and have been the basis of many confirmatory studies with a wide variety of sample types. QuEChERS has been applied to virtually all of the pesticide target analytes previously listed (Hercegová et al. 2006; Payá et al. 2007; Kirchner et al. 2008; Kmellár et al. 2008; Kovalczuk et al. 2008; Kruve et al. 2008; Lesueur et al. 2008; Mol et al. 2008, 2012; Nguyen et al. 2008; Schenck et al. 2008; Jiang et al. 2008; Kowalski et al. 2010; Lacina et al. 2010, 2012; Lehotoy et al. 2010; Mastovska et al. 2010; Wong et al. 2010; Chung and Chan 2010; Gilbert-López et al. 2010, 2012; Koesukwiwat et al. 2010, 2011; Zhao et al. 2011; Liu et al. 2011; Romero-González et al. 2011; Chen et al. 2011, 2012a, 2012b, 2013; Zhang et al. 2011; Park et al. 2011; Kittlaus et al. 2011; Dasika et al. 2012; Chung and Lam 2012; Filho et al. 2012; Fernandez et al. 2012; Cervera et al. 2012; Garrido-Frenich et al. 2012; Geis-Asteggiante et al. 2012; Kwon et al. 2012; Lozano et al. 2012; Rajski et al. 2013; Chamkasemn et al. 2013; Kaewsuya et al. 2013; Hou et al. 2013).

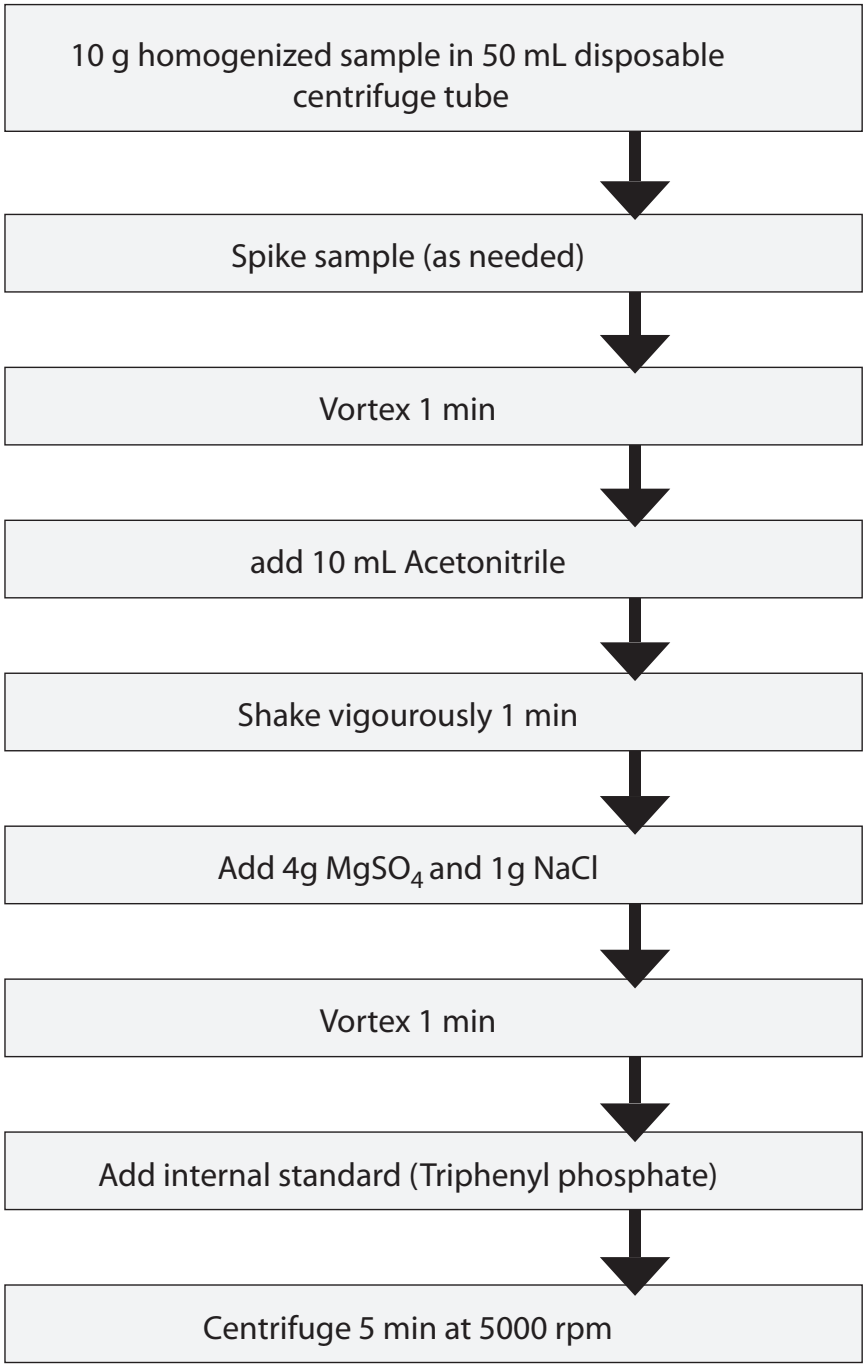


Figure 2. Sample extraction using Bond Elut Kit for QuEChERS sample preparation. Adapted from Usher and Majors (2012).

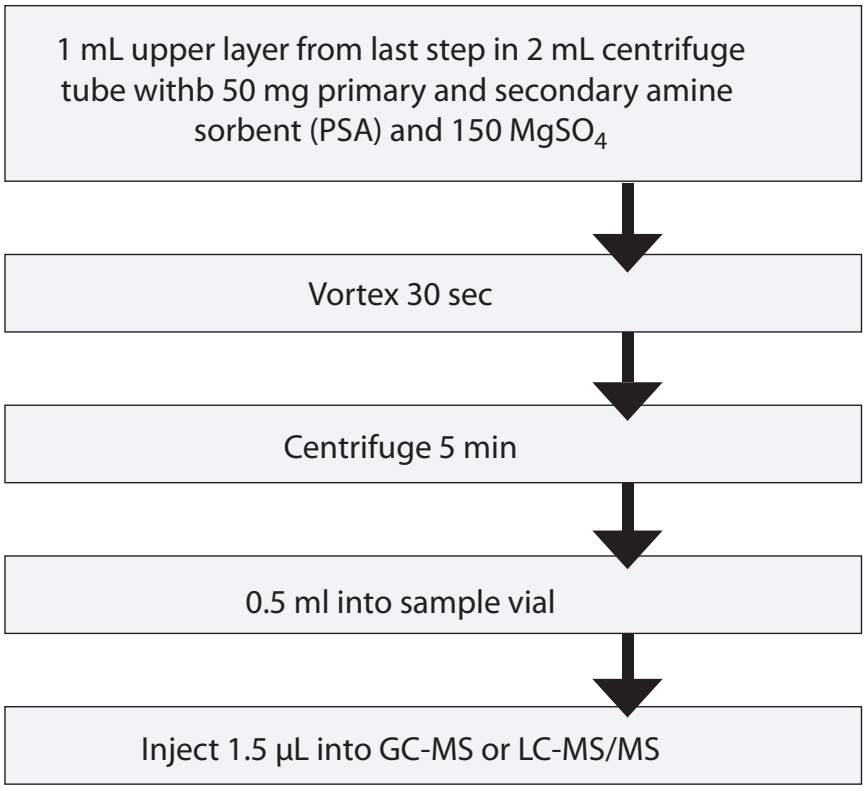


Figure 3. Dispersive SPE cleanup for QuEChERS sample preparation. Adapted from Usher and Majors (2012).

Figure 4 shows a representative chromatogram showing the typical analyte coverage by QuEChERS combined with an LC-MS/MS analysis.

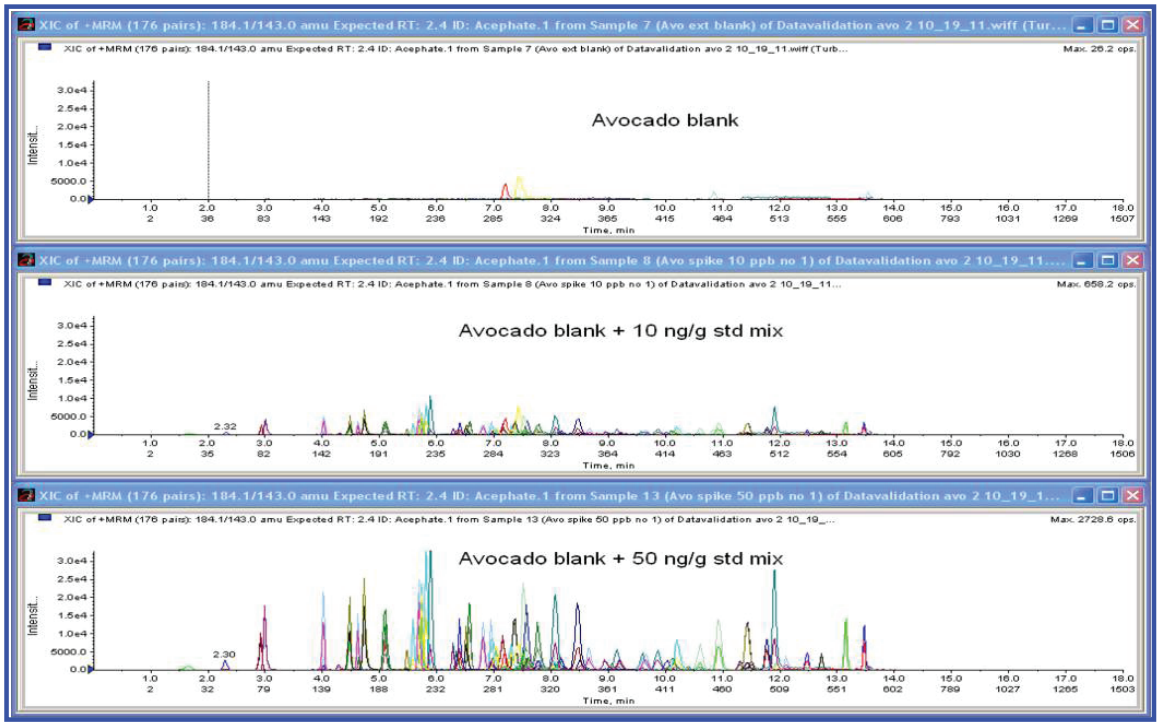


Figure 4. Reconstructed LC-MS/MS chromatogram of avocado blank, avocado blank spiked at 10 ng/g and 50 ng/g standard mix. The sample concentration is 0.12g sample/mL solvent, with 1 µl injection volume. From Chamkasem et al. 2013.

B. Analytical methods: Chromatography

After a sample has been extracted and interferences removed, the individual target compounds must be separated from one another, identified, and measured. Chromatography comprises a group of analytical methods that include separation, identification and measurement steps, which have become highly sensitive and automated. The various subtypes of chromatography – thin-layer (TLC), gas chromatography (GC), and liquid chromatography (LC) and high-performance liquid chromatography (HPLC) – differ primarily in the mechanism of compound separation. In all cases a sample mixture is introduced to the separation stage and a mobile phase (flowing liquid solvent, or pressurized gas) moves the mixture into contact with a stationary phase that attracts sample components to varying degrees. As the mobile phase continues to move compounds, they become separated from one another due to the interaction with the stationary phase. As the run completes, the compounds are detected and measured.

Thin layer chromatography (TLC), though rarely used for pesticide residue analysis, is the simplest of modern chromatography techniques. The stationary phase is a thin layer (e.g.: 250 µm) of finely divided silica, alumina, cellulose or other porous solid sorbent, bound to the surface of a rigid glass, aluminum or plastic plate. Sample extracts are applied as a spot near one edge of the plate, and the plate is placed in a developing tank with a

shallow (~0.5 cm) pool of solvent, that wets the bottom edge of the plate, but does not reach to the dried sample spot. As capillary action draws solvent (the mobile phase) up the plate, sample compounds dissolve in the moving solvent, and are drawn in the direction of the capillary flow. The mobile phase stops flowing when it reaches the opposite edge of the plate, and compound movement stops. The plate is said to have "developed," and is removed from the solvent tank; now the compounds can be detected. In TLC, compounds are visualized on the plate itself. The plate can be exposed to ultraviolet light, and compounds are observed by fluorescence or fluorescence quenching. Reagents that initiate chemical reactions producing color changes are possible (Tanuja et al. 2007). Even enzymatic reactions have been utilized to detect insecticides that inhibit acetycholinesterase (Zoun and Spierenburg 1989). Density of spots can be measured by dedicated scanners or digital photography, and related to compound concentration. TLC has the advantage of being inherently parallel: many sample spots can be applied across a plate and developed at once for greater throughput. Numerous TLC methods for cannabinoid analysis have been reported, however, sensitivity at residue levels may be limiting.

Liquid chromatography (LC) and high-pressure liquid chromatography (HPLC) are very similar, but instead of using an open plate, sorbent is packed into a cylindrical column, and solvent is pumped through. LC and HPLC differ from one another by sorbent particle size, solvent flow rates, pump pressures, and applications. LC typically is used in lower-resolution preparative chromatography, while HPLC has become highly developed for analytical applications. In both methods the sample is usually introduced by switching a loop containing the sample into the solvent flow stream with a multiport valve. As the compounds emerge ("elute") from the column at the downstream end, they can pass through optical detectors (ultraviolet/visible light absorption cells, refractive index cells, diode array detector (DAD) cells, fluorescence cells, etc.), or be nebulized into a mass spectrometer. The latter "hyphenated" methodology will be discussed further below. With optical detection the elution time ("retention time") is the primary identifying parameter for each target, but with multi-wavelength detectors wavelength-specific absorption or absorbance spectra provide additional confirmatory information.

In gas chromatography (GC) volatile sample components are separated in a gas stream flowing through a capillary column (e.g.: 250 μm inside diameter) many meters in length. The inner walls of the column are coated with a polymer film stationary phase that differentially retards sample components, creating the separation. Samples are injected as liquid solutions into a heated injector, where the solvent and sample vaporizes. Note that non-volatile compounds cannot survive the heat of the injector port, and must be chemically modified prior to analysis through formation of volatile derivatives. For many large or fragile molecules GC is not suitable, and these must be analyzed with HPLC systems.

As sample components move into and finally elute from the column they can be sensed by a variety of detectors. Some of the most common types are the flame ionization detector (FID) and thermal conductivity detector (TCD), but in pesticide analysis element-specific detectors have been extensively used, such as the electron capture (ECD, sensitive to halogens), the nitrogen-phosphorous (NPD), and the flame photometric detectors (FPD,

for sulfur containing compounds). As the target compound enters the detector, ionization or background current quenching generates a voltage signal, dependent on the sample density, producing peaks above a relatively flat baseline. As with HPLC, these detectors rely on precise retention times for compound identification. Voltage peaks are integrated, leading to calibrations of voltage response against compound concentration.

Generally speaking, GC ionization detectors and single-wavelength UV absorbance instruments in LC are well suited to analytes present in part-per-million concentrations (relatively high levels), as long as they can be well separated, or resolved, from one another. Some of the element specific detectors in GC are sensitive into the low ppb range, or lower. However, optical detectors in LC and GC ionization detectors do not provide information to help identify a compound, other than the time of emergence from the separation stage (retention time) and the response type of the detector (e.g., an ECD response indicates detection of a halogenated compound at a particular retention time). This places a great burden on the analyst to compare sample peaks against reference standards to assure identity. Confirmation may involve parallel columns with separate detectors of different types, and instrument complexity multiplies.

To provide more selectivity of response and provide additional confirmational information, spectroscopic detectors were developed. These either exploited optical properties (continuous UV and visible light absorption spectra, resolved by a multiple wavelength array, as in the diode-array detector, or DAD) or, after ionization of target compounds, analysis of charged particles by a mass spectrometer (MS). While both approaches have been applied to gas chromatography (GC), HPLC, and most recently, TLC, the greatest strides have been made in development of GC-MS and HPLC-MS. Mass spectrometers produce a unique mass "fingerprint" for each compound that can be used to unequivocally identify a peak in a chromatogram. These technologies merit discussion in greater detail.

C. Chromatography with mass spectrometry

Using a mass spectrometer as a detector with chromatography methods can provide greater specificity of response and additionally confirm compound identity. Rather than simply identifying analytes visually as peaks with specific retention times, as in conventional chromatography, mass spectrometry techniques distinguish a compound's molecular contents according to their mass spectra. Once completed, mass spectrometry produces a plot of masses produced from a molecule or its fragments after ionization.

The spectrometer has three components: an ion source, a mass analyzer, and a detector. All components reside in a high vacuum. GC-MS is the simplest interface case: column effluent is directly introduced into the MS at the "ion source." The ion source provides a mechanism to ionize a portion of the molecules that enter it; in many GC-MS instruments this is a heated filament that ejects a stream of electrons, much like vacuum amplifier tubes of the 1940s. Electrons hitting molecules will knock off an electron, creating a positively charged ion with a molecular weight identical to the molecular weight of the original molecule (the "molecular ion"). This is referred to as electron ionization (EI).

Secondary collisions fragment the parent ion further at characteristic locations. A positively charged plate repels the ions toward the mass analyzer, a device that uses electrostatic fields to sort charged particles by their mass-to-charge ratio (m/z). The most common mass analyzers consist of a set of four rods (a quadrupole) with applied electrostatic fields that "scan" across masses of the generated particles, allowing only those of a given m/z ratio to pass through to the detector at any given instant. The detector (usually a semiconductor or related device) measures the instantaneous abundance of particles hitting it, and uses these events to generate a mass spectrum plot (Figure 1). Each molecule compatible with this analysis will generate a unique spectrum, and these can be compared rapidly against libraries of hundreds of thousands of spectra for known substances. In GC-MS an alternate ionization strategy entails primary ionization of a reagent gas such as isopropanol, that secondarily causes target molecules to be ionized; this "soft" ionization is called chemical ionization (CI), and results in much more abundant molecular ion peaks at the expense of less fragmentation.

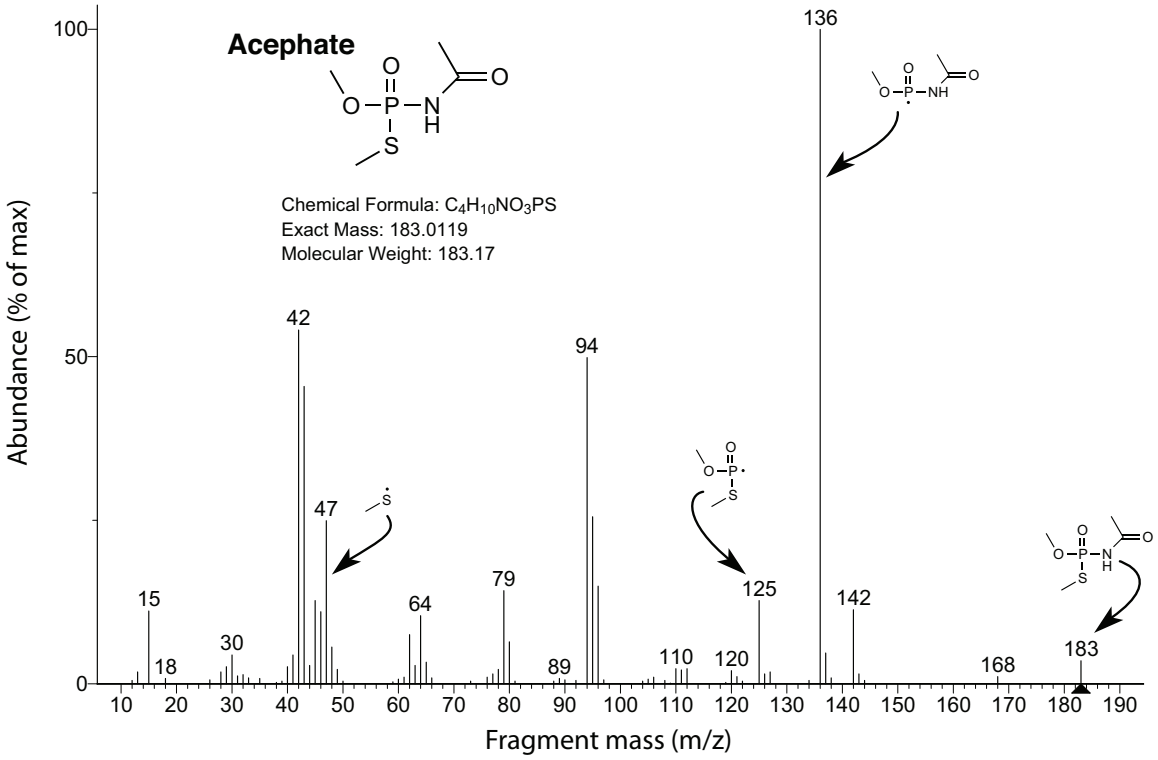


Figure 1. Electron-Impact (EI) mass spectrum for acephate, with partial assignment of fragment structures.

In HPLC-MS additional problems must be solved. The liquid solvent mixture of the mobile phase must be removed before target compounds enter the high vacuum of the MS. This is accomplished by a variety of techniques involving nebulizing, heated sheath gases, and high voltage gaps that impart charges to the surface of aerosolizing droplets. As solvent evaporates away, charge density increases, contributing to target compound

ionization and fragmentation. Ionized compounds are deflected into an inlet, and are segregated by their m/z ratios as described above. Despite the apparent additional complexity, HPLC-MS is the most desirable approach for multi-residue analysis owing to its ability to detect non-volatile and heat labile compounds without chemical pretreatment.

If compounds are overlapped (incompletely resolved), each will contribute masses to the resulting spectrum. Great effort has been applied to reducing this problem in GC-MS through extensive pre-purification of samples (clean up) prior to analysis, and through development of highly efficient columns. Interference by co-eluting compounds in both GC-MS and LC-MS is also addressed by adding additional mass analyzer stages in series (e.g.: a tandem mass spectrometer, MS/MS). One common configuration is the triple quadrupole, with two mass analyzers in series, separated by a non mass-resolving quadrupole that acts as a collision cell. This center cell contains a low pressure inert gas such as argon, helium, or nitrogen which induces fragment dissociation by collision. Only known masses are admitted into this region (and further fragments result) which are passed through the second mass-resolving quadrupole to the detector. Through different modes of operation structural details can be deduced, and background interferences rejected, producing great sensitivity and selectivity.

Other MS technologies are emerging with different capabilities and strengths, such as ion trap instruments, time-of-flight (TOF) systems, and hybrid systems (e.g.: Q-TOF types, that follow a quadrupole with a flight chamber). Some of these are capable of extremely high mass accuracy, further increasing confidence of compound identification. Allied to the technologies already described, these provide higher accuracy of mass estimation, sufficient to probe isotopic distribution, without resorting to sequential stages of ionization. These instruments offer some advantages over tandem mass spectrometry, or MS/MS. MS/MS techniques depend on targeted analysis with *a priori* knowledge of target retention time and structure in order to program windows during an analysis when masses are selected (by the first quadrupole) for secondary collision (in the second, non-mass-resolving collision quadrupole) and mass analysis following the third quadrupole stage. Within the acquisition window MS/MS systems have unparalleled selectivity, efficiently rejecting molecules that do not match the programmed mass selection. Within that window metabolites, minor components and unknowns will be rejected (i.e.: not detected at all), and there is no way to retrospectively reprocess chromatographic data of this type to retrieve information missed by the initial data acquisition. The TOF and ion trap designs circumvent this limitation, and potentially allow extensive investigation of historical data gathered using these instruments. These are relatively new additions to the field and have only recently been critically evaluated against more established MS/MS instruments (Pico et al. 2009; Thurman et al. 2009; Mastovska et al. 2010; Kaufmann et al. 2012; Polgár et al. 2012). They also have lower precision at low analyte concentrations, so for critical quantitative work MS/MS systems are preferred (Jacob 2013).

MS/MS has been extensively applied to pesticide analysis since the mid-2000s, with both GC-MS/MS (Garrido-Frenich et al. 2005, 2007; Okihashi et al. 2007; Aguado et al. 2007; Payá et al. 2007; Lee et al. 2008; Du et al. 2012; Mastovska and Wylie 2012; Chen et al. 2013; Rajski et al. 2013; Hou et al. 2013) and LC-MS/MS technologies applied (Agüera et al. 2004; Ortelli et al. 2004; Alder et al. 2004; Hernández et al. 2006; Ferrer et al. 2007;

Lehotay 2007; Leandro et al. 2007; Mol et al. 2007, 2008; Payá et al. 2007; Garrido-Frenich et al. 2008; Kovalczuk et al. 2008; Hengel and Miller 2008; García-Reyes et al. 2009; Huang et al. 2009; Drozdzynski and Kowalska 2009; Mayer-Helm 2009; Camino-Sánchez et al. 2010; Jia et al. 2010; Riedel et al. 2010; Kanrar et al. 2010; Ferrer Amate et al. 2010; Wong et al. 2010; Chung and Chan 2010; Fillatre et al. 2010, 2011; Gilbert-López et al. 2010; Lu et al. 2010; Liu et al. 2011; Romero-González et al. 2011; Chen et al. 2011, 2012a, 2012b, 2013; Kmellár et al. 2011; Kruve et al. 2011; Sack et al. 2011; Lee et al. 2011; Zhang et al. 2011; Kittlaus et al. 2011, 2013; Wang et al. 2011; Chung et al. 2012; Jiang et al. 2012; Fornal and Stachniuk 2012; Núñez et al. 2012; Polgár et al. 2012; Hollosi et al. 2012; Lacina et al. 2012; Rajski et al. 2013). Most of the aforementioned papers describe multi-residue and multi-class analyses, addressing up to hundreds of compounds in a single method, and many have addressed compounds of concern identified in this study, as show in Table 5.

Table 5. Initial survey compounds of concern treated in the reviewed literature.

Primary Active Ingredient	ELISA	TLC	GC-ECD	GC-FPD	GC-MS, GC-MS/MS	HPLC-DAD	HPLC-ESI-MS, MS/MS	HPLC-Ion Trap, HPLC-TOF
acephate		√			√		√	√
acequinocyl						√		√
avermectin						√	√	√
azadirachtin		√					√	√
bifenazate							√	√
daminozide							√	√
diazinon		√			√		√	√
ethephon		√						
etoxazole			√	√	√	√	√	√
fenoxycarb	√	√	√	√	√	√	√	√
imazalil					√		√	√
imidacloprid	√						√	√
myclobutanil			√		√	√	√	√
paclobutrazol			√	√	√		√	√
propoxur		√	√	√	√		√	√
pyrethrins					√	√	√	√
spinosyn							√	√
spiromesifen					√		√	
carbendazim					√		√	√

The confidence resulting from combining mass spectrometry with efficient chromatography and modern data systems makes GC-MS, and more particularly HPLC-MS, the methods of choice for multi-residue pesticide analysis. Steep Hill Laboratories currently has a single quadrupole GC-MS with parallel FID for general cannabinoid and terpene analysis and a single quadrupole HPLC system also used for cannabinoid and terpene analyses and some pesticide measurements, and is planning to acquire a triple quadrupole HPLC system (HPLC-MS/MS) for greater capability in low-level residue applications.

VI. Microbial Monitoring

A. Sample preparation

For both counting of total microbial numbers and subsequent identification, samples are first gently mixed with water. Weighed samples are placed in plastic bags with a known volume of water and placed in a Stomacher® paddle blender which gently kneads the bag and liberates the microflora. A 1 mL aliquot of the water suspension is pipetted to dilution flasks with a sterile pipette tip, and three 10X dilutions are made with distilled water (i.e.: making 1/10, 1/100 and 1/1000th the initial suspension concentration). Triplicate plates are inoculated with liquid from each dilution level for incubation and counting.

B. Enumeration

It is important to be able to determine the total amount of microbial contamination present on a *Cannabis* sample to verify the sample is safe for human consumption. Methods for microbial enumeration are laid out by the U.S. Pharmacopeia in USP 2021 and USP 61, and Steep Hill Laboratories follows this strategy. Following serial dilution, samples aliquots are plated out on Sabouraud Dextrose Agar media. Three replicated samples are run at each dilution level to obtain an accurate count of mold and bacterial numbers present. Plate count methods use arithmetic mean counts of colonies to calculate CFU per gram for the original sample. Limitations of this method include inhibition of fungal or bacterial growth on the media plates by compounds present in the ground plant sample. This may result in lower counts for the total combined yeast and mold count than are actually present on the sample. To account for this recovery, substances that neutralize the inhibitory substances can be added to the media to obtain more accurate counts according to USP 2021. As mentioned earlier, THC and CBD have been shown to inhibit growth of fungi other than *Penicillium* and *Aspergillus* (Dahiya & Jain 1977), but presumably these compounds would not be present in the aqueous medium used to prepare the initial extract. Steep Hill Laboratories uses the microbial contamination guidance thresholds of the American Herbal Products Association (AHPA 2012), as follows:

(i) for dried, unprocessed herbs for use as ingredients in dietary supplements, and (ii) for herbal supplements in solid form consisting of dried, unprocessed herbs:

- Total aerobic plate count: 10^7 colony forming units/gram
- Total yeasts and molds: 10^5 colony forming units/gram
- Total coliforms: 10^4 colony forming units/gram
- *Salmonella* spp.: not detected in 25 grams
- *Escherichia coli*: not detected in 10 grams

C. Identification methods

After estimation of microbial density, it may be necessary to further identify the organisms detected. Macroscopic and microscopic examination of colonies from enumeration plates often gives a reasonably reliable initial identification. If it is deemed important to produce a more rigorous characterization, other techniques must be applied.

Polymerase chain reaction (PCR) is a widely used method for determining the identity of species of both plants and fungi by DNA sequencing. PCR utilizes DNA polymerase enzymes to replicate (amplify) small amounts of DNA from a sample so that there is sufficient material for further analysis. Samples of leaf are prepared for extraction of fungal DNA by either the NaOH extraction protocol of Wang et al. (1993) or a DNA extraction kit. Primers, short sequences of DNA added to the sample to facilitate the polymerase reaction, attach to certain points on a DNA strand and can be designed to specifically attach to sequences specific to a genus or species of concern such as *Aspergillus fumigatus*. Amplified DNA is then cut into shorter strands with restriction enzymes (enzymes that bind to, and cut, DNA at specific locations), which can be separated by gel electrophoresis. Presence of a band on a gel would indicate a positive test for a particular species and the presence of that species in the sample. Different tests have to be run to detect each species; thus a regimen for fungal testing will be more expensive with the addition of each fungal species or pathogen.

When identifying unknown fungi, general fungal primers can be used to amplify the DNA, such as the ITS1f region (Gardes 1993) and ITS4 (White 1990); the amplified segments can then be cleaved and sequenced. This is only possible when a pure culture of the questionable fungi is available (this may require subculturing from enumeration plates). DNA sequences can then be identified by comparison to reference sequences such as those found on GenBank. High throughput DNA sequencing and "real time" quantitative PCR (Haugland 2004, 2007; Nonnenmann 2012; Vesper 2011) are relatively new methods of DNA testing that allow testing of single samples for multiple species of fungi. General fungal primers are used and test results would come back with all fungal species present on that sample, including endophytes and airborne spores that may have happened to land on the sample. DNA sequencing can also be of use, by allowing detection of fungi that are not culturable. At present these genetic methods are the most comprehensive test available, but also very expensive and not feasible for most labs.

PCR also has its limits. Most PCR reactions require a significant amount of the particular fungal DNA in question to be present for the primers and the PCR to detect and amplify it. (The exception is quantitative PCR.) So negative test results may not confirm that the particular fungal pathogen in question is not present on the sample. It also does not address the amount of fungi present on the sample or the level of potentially harmful mycotoxins present on the sample.

D. Mycotoxins in Cannabis

Mycotoxins are toxic metabolic products produced by fungi. Usually this term is applied to compounds produced by molds inhabiting crops or harvested products. Some mycotoxins are specific to a particular mold, while others may be produced by a number of different species. The role of mycotoxins in the life of fungi in most cases remains unknown, although they presumably alter the external environment to aid fungal growth. An example is penicillin inhibiting bacterial growth near *Penicillium* colonies. Over 100 countries have established regulatory limits for mycotoxins in food and animal feeds, but progress in implementing monitoring programs has been slowed by lack of appropriate analytical methods in some countries (van Egmond et al. 2007).

ELISA (Enzyme Linked ImmunoSorbent Assay) is a type of test that detects target analytes using antibodies (small, immunologically active proteins that bind to foreign materials, or antigens) produced by a host (e.g., rabbit) that has been exposed to the target substance. Particularly with small molecules, the antibody response is not triggered in the host by the native compound. In these cases a larger molecule (usually a protein) is bonded to the target, and numerous antibodies are generated to the target-protein complex. Some of these antibodies will successfully bind to the target alone, and these become useful as detection tools. The sample is bound to a well in a plate, and then antibody (e.g.: bound to an enzyme capable of catalyzing a reaction forming a colored product) is added, then the plate is rinsed to remove antibody that did not bind to a target (e.g., a mycotoxin). Finally, a substrate is added, and if antibody has been immobilized by binding to a target molecule, detectable colored products are formed. ELISA been shown to be very effective in identifying the presence of mycotoxins (Scott 1976). ELISA tests are specific to one mycotoxin. So, testing for numerous mycotoxins of health involves a battery of specific antibody-enzyme complexes. This can become quite expensive since each individual test costs from $25 to $50 per target (Carlson & Ensley 2003).

There are several drawbacks of ELISA tests. Some tests can identify the presence of a particular mycotoxin, but cannot quantify how much is present. Further, it is important to take either a single sufficiently large sample or many small samples, in order to ensure the representativeness of the sample. For most ELISA tests, a mycotoxin detected at very small levels may still be reported as a "negative"; so a negative result does not necessarily demonstrate an absence of the target mycotoxins. Lastly, it is important to remember that ELISA tests can only detect mycotoxins chosen as target analytes; as the old adage goes, what you seek is what you find.

High performance liquid chromatography (HPLC) and thin-layer chromatography (TLC) are both reliable methods for identifying the presence of mycotoxins (el-Maghraby & Abdel-Sater 1993). Much research and standardization has been done in this field and many reference standards exist for both mycotoxins and other fungal metabolites that may be harmful to the health of those consuming them (Frisvad 1987). HPLC with fluorescence detection is effective for many mycotoxins. For pesticide residues, HPLC-MS techniques are much more powerful and can provide confirmation in the mass spectra of detected compounds.

VII. Heavy Metals

A. Sample preparation

Metals are generally detected with optical or mass spectrometric techniques as described below. In all cases samples are digested in mineral acids to eliminate organic interferences, and the resulting solutions nebulized into the instrument for analysis. Today, sample digestion is often performed in closed vessels with strong acid, with temperature and pressure raised with microwave irradiation ("microwave digestion").

B. Instrumentation

Suitable approaches for metals analysis include instruments such as atomic emission spectroscopy (AES), atomic absorbance spectroscopy (AAS), or the related inductively

coupled plasma-mass spectroscopy (ICP-MS). All of these are highly sensitive, capable in some cases of part-per-trillion measurements, to applicability to even very low-level measurements is unquestionable. However, these are expensive instruments that may be out of reach for general purpose *Cannabis* analytical laboratories. Methods for metals analysis (primarily focusing on food analysis) are presented in detail in the FDA Elemental Analysis Manual (FDA 2013a), which summarizes all aspects of sample preparation, instrument calibration and data reduction.

A new approach that both lowers instrumentation cost and speeds analysis is X-ray fluorescence (XRF). This technology has the advantages of non-reliance on highly processed samples, and in some cases, portability. At least one manufacturer is now targeting the medical *Cannabis* market with a handheld device; claimed limits of detection are given in Table 6.

Table 6. Manufacturer's reported limits of detection (LOD), in parts per million, for metals of concern in *Cannabis*. Data for the Olympus DELTA Premium 3-Beam Soil Ta/Tu Tube, SDD handheld XRF instrument.[1]

Element	LOD	Element	LOD
P	500-700	**Se**	1-3
S	100-250	**Sr**	1-3
Ti	7-15	**Cd**	6-8
Cr	5-10	**Sn**	11-15
Ni	10-20	**Sb**	12-15
Cu	5-7	**Hg**	2-4
Zn	3-5	**U**	2-4
As	1-3	**Pb**	2-4

[1]http://www.olympus-ims.com/en/applications/potential-toxins-medical-marijuana-use/. Accessed 12 September, 2013.

VIII. Pests and other foreign matter

As discussed earlier, the FDA does not consider insect contamination or contamination by other extraneous debris to be health hazards of concern. Nonetheless, the presence of these materials reduces the product's perceived quality, so producers, distributors, and retailers may desire to rate their products for these factors. Our laboratory experience does not include pest contamination, again owing to the *Cannabis* products submitted for analysis to date, headed into the medical *Cannabis* market. That said, there are guideline FDA Microanalytical Procedures Manuals (MPM; FDA 2013b) for food testing that will be valuable resources for developing standards for contamination if it becomes necessary in the *Cannabis* industry.

PART THREE - CONCLUSIONS

VI. Recommendations

Pesticide Use and Residue Monitoring

We have emphasized the requirements for trace residue analysis appropriate to support pesticide monitoring for the regulated *Cannabis* market in Washington State.

- The Board is encouraged to communicate the need for formal pesticide registration by the state agricultural department to help deliver rational pest control advice to growers as soon as possible. We feel it's likely that, particularly in the early years of legal *Cannabis* cultivation, specific and sound recommendations for pest control in this crop will be few, and excessive use of chemical pesticides may take place. Guidelines from the EPA management of pesticide use in other crop types are numerous and specific. Emerging European residue standards for tobacco, the only other product intended for smoking, provide useful goals to meet or exceed when considering the need for, and magnitude of, residue tolerance limits for *Cannabis*.
- The Board should encourage laboratories to conference extensively with established analysts and carefully review the scientific literature to review instrumentation developments and critical comparative studies prior to dedicating capital to one or another of the available instrumentalities. Laboratories authorized to analyze I-502-regulated *Cannabis* for pesticide residues will need to demonstrate competence with multi-residue analyses and modern instrumentation. Although GC-MS/MS seems a logical and somewhat lower cost methodology, recent reports indicate that precision and sensitivity requirements with this technology are more easily met by LC with tandem MS detection (HPLC-MS/MS), particularly if the number of required target analytes is high (Alder et al. 2006).
- Method development and validation for pesticide analysis in *Cannabis* products will have to be performed, and should be published in peer-reviewed literature for early contributions to a subject essentially absent from today's science.

Contamination by Fungi and Bacteria

Though no microbial contamination standards exist for marijuana in particular, foods and herbal products have long been subject to threshold tolerance regulations. It will be important to construct similar standards for marijuana, such that all retailers and testing facilities will be able to comply and customers may be confident in product safety.

- Microbial testing can be satisfactorily achieved with enumeration (CFU counts). This method is commonly available at *Cannabis* laboratories, and comes at lower costs than more rigorous identifications of fungi and bacteria, such as those utilizing genetic tests. However, enumeration is not appropriate for plant disease analysis.
- Mycotoxins and known harmful pathogens should not be allowed at any level.
- Harmful fungi can be identified by coupling HPLC and MSQPCR methods. Mold specific quantitative PCR (MSQPCR) is the most reliable method available to identify

potentially harmful fungi, but it cannot quantify the amount of mycotoxins present. Quantification can be performed separately with HPLC or HPLC-MS/MS, as required.

- These methodologies may require some laboratories to make significant investments in equipment and training, but will provide the general public with the most comprehensive and reliable data.

Metals, Pests and Other Foreign Matter

Heavy metals have already been detected in illicit marijuana and can pose a grave health threat to users. It will be important for the regulated marketplace to reduce this risk without excessive expense. It is very costly to detect heavy metals in the finished product, due to the nature of highly sensitive spectroscopic techniques. A suitable approach might involve a quality control inspection program that instead focuses on production process and intermediary outcomes.

- Hand-held X-ray fluorescence devices might possibly be deployed as field monitoring devices, perhaps at distribution points, in order to provide economical screening within the supply chain.
- Monitoring production practices might require significant ongoing effort, depending on the details of the program.

Preventative methods

The medical *Cannabis* industry in California is uniquely labor-intensive; owner-growers are in near constant contact with crops and field conditions, making the enterprise ideally suited for adoption of Integrated Pest Management (IPM) practices. According to IPM, physical and biological factors contributing to pest or disease problems are routinely evaluated and manipulated to avoid dependence on toxic chemicals.

- Exploitation of IPM in Washington's larger scale recreational *Cannabis* production would lessen the likelihood of problematic pesticide residues emerging, and a rigorous monitoring program could ensure confidence that the product is both known and safe (McPartland et al. 2000; Rosenthal 2012).

Finally, there are severe gaps in the knowledge about *Cannabis*, since formal agricultural research hasn't been permitted for this crop in nearly 100 years. The State of Washington is strongly encouraged to continue to work with horticulturalists, entomologists, plant pathologists, toxicologists, and food scientists to address the information needs of this new industry. Encouraging scientific scrutiny, research, and technological development are critical steps to ensuring safe and sustainable production.

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APPENDIX 1. GUIDANCE RESIDUE LEVELS FOR CROP PROTECTION AGENTS, CORESTA, JULY 2013.

Appendix Table 1. Guidance residue levels (GRLs) for crop protection agents, reproduced from CORESTA Guide No. 1, July 2013 (**Not** a recommended list of CPAs for tobacco, but representative for analytical residue targets as of the publication date).

Primary Active	GRL	Residue definition, Notes
2,4,5-T	0.05	2,4,5-T
2,4-D	0.20	2,4-D
Acephate	0.10	Acephate
Acetamiprid	2.50	Acetamiprid
Acibenzolar-*S*-methyl	5.00	Acibenzolar-S-methyl
Alachlor	0.10	Alachlor
Aldicarb (!)	0.50	sum of Aldicarb, Aldicarb sulfoxide and Aldicarb sulfone, expressed as Aldicarb
Aldrin + Dieldrin	0.02	Aldrin + Dieldrin
Azinphos-ethyl	0.20	Azinphos-ethyl
Azinphos-methyl	0.30	Azinphos-methyl
Benalaxyl	2.00	Benalaxyl
Benfluralin	0.06	Benfluralin
Benomyl [a]		sum of Benomyl, Carbendazim, and Thiophanate-methyl expressed as Carbendazim; see Carbendazim
Bifenthrin	2.50	Bifenthrin
Bromophos	0.04	Bromophos
Butralin	5.00	Butralin
Camphechlor (Toxaphene)	0.30	Camphechlor (mixture of chlorinated camphenes)
Captan	0.70	Captan
Carbaryl	0.50	Carbaryl
Carbendazim [a]	2.00	sum of Benomyl, Carbendazim, and Thiophanate-methyl
Carbofuran (!)	0.50	sum of Carbofuran and 3- Hydroxycarbofuran expressed as Carbofuran
Chinomethionat	0.10	Chinomethionat
Chlorantraniliprole	10.00	Chlorantraniliprole
Chlordane (!)	0.10	sum of cis-Chlordane and trans- Chlordane
Chlorfenvinphos (!)	0.04	sum of (*E*)-Chlorfenvinphos and (*Z*)-Chlorfenvinphos
Chlorothalonil	2.00	Chlorothalonil
Chlorpyrifos	0.50	Chlorpyrifos
Chlorpyrifos-methyl	0.20	Chlorpyrifos-methyl
Chlorthal-dimethyl	0.50	Chlorthal-dimethyl
Clomazone	0.20	Clomazone
Cyfluthrin (!)	2.00	Cyfluthrin (sum of all isomers)
Cyhalothrin (!)	0.50	Cyhalothrin (sum of all isomers)
Cymoxanil	0.10	Cymoxanil
Cypermethrin (!)	1.00	Cypermethrin (sum of all isomers)
DBCP	0.05	DBCP (1,2-dibromo-3- chloropropane)
DDT (!)	0.20	sum of o,p'- and p,p'-DDT, o,p'- and p,p'-DDD (TDE), o,p'- and p,p'-DDE expressed as DDT
Deltamethrin [b]	1.00	sum of Deltamethrin and Tralomethrin expressed as Deltamethrin

Primary Active Ingredient	GRL (ppm)	Residue definition, Notes
Demeton-*S*-methyl (∑)	0.10	sum of Demeton-*S*-methyl, Oxydemeton-methyl (Demeton-*S*-methyl sulfoxide) and Demeton-*S*-methyl sulfone expressed as Demeton-*S*-methyl
Diazino	0.10	Diazinon
Dicamba	0.20	Dicamba
Dichlorvos [c]	0.10	sum of Dichlorvos, Naled and Trichlorfon expressed as Dichlorvos
Dicloran	1.00	Dicloran
Diflubenzuron	0.10	Diflubenzuron
Dimefox	0.01	Dimefox
Dimethoate [d]	0.05	sum of Dimethoate and Omethoate expressed as Dimethoate
Dimethomorph (∑)	2.00	sum of (*E*)-Dimethomorph and (*Z*)-Dimethomorph
Dinocap (∑)	0.60	sum of Dinocap isomers and 0.60 Dinocap phenols expressed as Dinocap. Currently, Dinocap isomers expressed as Dinocap ([illegible]) because Dinocap phenols standard is not available. Dinocap phenols should be also expressed as Dinocap ([illegible]) when standard will be available.
Diphenamid	0.05	Diphenamid
Disulfoton (∑)	0.10	sum of Disulfoton, Disulfoton sulfoxide, and Disulfoton sulfone expressed as Disulfoton
Dithiocarbamates [e] (as CS_2)	5.00	Dithiocarbamates expressed as CS_2. In countries where fungal diseases such as blue mould are a persistent problem in the field throughout the growing season, the use of dithiocarbamates (DTC) fungicides may be an essential part of the season-long disease management strategy and in keeping with GAP as a means of ensuring crop quality and economic viability for the producer. Under high disease pressure residues of dithiocarbamates (DTC) fungicides slightly in excess of the specified GRL may be observed. In countries where there is not a field fungal disease problem the use of fungicides is not necessary, and there should be no residues detected. Consistent with GAP, dithiocarbamates (DTC) fungicides must be used only according to label instructions to combat fungal diseases in the seedbed and in the field.
Endosulfans (∑)	1.00	sum of alpha- and beta-isomers and Endosulfan-sulphate expressed as Endosulfan
Endrin	0.05	Endrin
Ethoprophos	0.10	Ethoprophos
Ethylene dibromide	0.05	Ethylene dibromide
Famoxadone	5.00	Famoxadone
Fenamiphos (∑)	0.50	sum of Fenamiphos, Fenamiphos sulfoxide and Fenamiphos sulfone expressed as Fenamiphos
Fenchlorphos	0.04	Fenchlorphos
Fenitrothion	0.10	Fenitrothion
Fensulfothion	0.04	Fensulfothion
Fenthion (∑)	0.10	sum of Fenthion, Fenthion sulfoxide and Fenthion sulfone expressed as Fenthion
Fenvalerate (∑)	1.00	Fenvalerate (sum of all isomers including Esfenvalerate)
Fluazifop-butyl (∑)	1.00	Fluazifop-butyl (sum of all isomers)

Primary Active Ingredient	GRL (ppm)	Residue definition, Notes
Flucythrinate (∑)	0.15	Flucythrinate (sum of all isomers)
Flumetralin	5.00	Flumetralin
Folpet	0.20	Folpet
Fonofos (∑)	0.05	Fonofos (sum of all isomers)
Formothion	0.05	Formothion
HCH (**α**-, ***β***-, **γ**-)	0.05	HCH (**α**-, ***β***-, **γ**-)
HCH (**γ**-) (Lindane)	0.05	HCH (**γ**-) (Lindane)
Heptachlor (∑)	0.02	sum of Heptachlor and two Heptachlor epoxides (cis- and trans-) expressed as Heptachlor
Hexachlorobenzene	0.02	Hexachlorobenzene
Imidacloprid	5.00	Imidacloprid
Indoxacarb (∑)	15.00	Sum of *S*- isomer + *R*- isomer
Iprodione (∑)	0.25	sum of Iprodione and N-3,5- dichlorophenyl-3-isopropyl-2,4-dioxoimidazolyzin-1- carboxamide expressed as Iprodione
Isopropalin	0.07	Isopropalin
Malathion	0.50	Malathion
Maleic hydrazide	80.00	Maleic hydrazide (free and bounded form). In some instances, where GAP is implemented and label recommendations with regard to application rates and timing are strictly adhered to, residue levels may exceed the current GRL of 80 ppm as a result of limited rainfall and the current technology available for application. However, as with all CPAs, all efforts should be made to strictly follow label application rates, and use should be no more than necessary to achieve the desired effect.
Metalaxyl (∑)	2.00	sum of all isomers including Metalaxyl-M / Mefenoxam
Methamidophos	1.00	Methamidophos
Methidathion	0.10	Methidathion
Methiocarb (∑)	0.20	sum of Methiocarb, Methiocarb sulfoxide, and Methiocarb sulfone expressed as Methiocarb
Methomyl [f]	1.00	sum of Methomyl, Methomyl- oxim, and Thiodicarb expressed as Methomyl
Methoprene	1.00	Methoprene
Methoxychlor	0.05	Methoxychlor
Mirex	0.08	Mirex
Monocrotophos	0.30	Monocrotophos
Naled [c]	see➛	sum of Dichlorvos, Naled, and Trichlorfon expressed as Dichlorvos; see Dichlorvos
Nitrofen	0.02	Nitrofen
Omethoate [d]	see➛	sum of Dimethoate and Omethoate expressed as Dimethoate; see Dimethoate
Oxadixyl	0.10	Oxadixyl
Oxamyl	0.50	Oxamyl
Parathion (-ethyl)	0.06	Parathion
Parathion-methyl	0.10	Parathion-methyl
Pebulate	0.50	Pebulate
Penconazole	1.00	Penconazole

Primary Active Ingredient	GRL (ppm)	Residue definition, Notes
Pendimethalin	5.00	Pendimethalin
Permethrin (∑)	0.50	Permethrin (sum of all isomers)
Phorate	0.10	Phorate
Phosalone	0.10	Phosalone
Phosphamidon (∑)	0.05	Phosphamidon (sum of *E*- and *Z*- isomers)
Phoxim	0.50	Phoxim
Piperonyl butoxide	3.00	Piperonyl butoxide
Pirimicarb	0.50	Pirimicarb
Pirimiphos-methyl	0.10	Pirimiphos-methyl
Profenofos	0.10	Profenofos
Propoxur	0.10	Propoxur
Pymetrozine	1.00	Pymetrozine
Pyrethrins (∑)	0.50	sum of Pyrethrins 1, Pyrethrins 2, Cinerins 1, Cinerins 2, Jasmolins 1 and Jasmolins 2
Tefluthrin	0.10	Tefluthrin
Terbufos (∑)	0.05	sum of Terbufos, Terbufos sulfoxide and Terbufos sulfone expressed as Terbufos
Tetrachlorvinphos	0.10	Tetrachlorvinphos
Thiamethoxam	5.00	Thiamethoxam
Thiodicarb [f]	see➛	sum of Methomyl, Methomyl- oxim, and Thiodicarb expressed as Methomyl; see Methomyl
Thionazin	0.04	Thionazin
Thiophanate-methyl [a]	see➛	sum of Benomyl, Carbendazim, and Thiophanate-methyl
Tralomethrin [b]	see➛	sum of Deltamethrin and Tralomethrin expressed as Deltamethrin;
Trichlorfon [c]	see➛	sum of Dichlorvos, Naled, and Trichlorfon expressed as
Trifluralin	0.10	Trifluralin
Vamidothion (∑)	0.05	sum of Vamidothion, Vamidothion sulfoxide and Vamidothion

[a] Carbendazim is the degradation product of Benomyl and Thiophanate-methyl. In the case the same sample contains residues of both Carbendazim and/or Benomyl/Thiophanate-methyl, the sum of the residues should not exceed 2.00 ppm.

[b] Deltamethrin is the degradation product of Tralomethrin. In the case the same sample contains residues of both Deltamethrin and Tralomethrin, the sum of the two residues should not exceed 1.00 ppm.

[c] Dichlorvos is the degradation product of Naled and Trichlorfon. In the case the same sample contains residues of both Dichlorvos and/or Naled/Trichlorfon, the sum of the residues should not exceed 0.10 ppm.

[d] Omethoate is the degradation product of Dimethoate. In the case the same sample contains residues of both Dimethoate and Omethoate, the sum of the two residues should not exceed 0.50 ppm.

[e] The Dithiocarbamates Group includes the EBDCs: Mancozeb, Maneb, Metiram, Nabam and Zineb – as well as Amobam, Ferbam, Policarbamate, Propineb, Thiram and Ziram.

[f] Methomyl is the degradation product of Thiodicarb. In the case the same sample contains residues of both Methomyl and Thiodicarb, the sum of the two residues should not exceed 1.00 ppm.