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Determination of eighty-two pesticides and application to screening pesticides in cannabis growing facilities

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ABSTRACT

Determination of pesticides in cannabis facilities is increasingly important as medicinal and recreational uses of cannabis products expand rapidly. We report a method involving wipe sampling, liquid chromatography separation, and tandem mass spectrometry, which enables determination of 82 pesticides out of the 96 regulated by Health Canada. To demonstrate an application of the method, we sampled and measured pesticides in two cannabis growing facilities, representing a non-certified and a certified site. We detected 41 pesticides in surface wipe samples at the non-certified site and 6 at the certified site. This study provides the first evidence showing pesticide occurrence on common surfaces in cannabis growing facilities and points to a need for routine monitoring and strict control of pesticide use in cannabis facilities.

Introduction

Cannabis has been used historically for a variety of purposes, the most common being medicinal and recreational (Andre et al., 2016; Zuardi, 2006; Mercuri et al., 2002; Touw, 2018). Medicinal benefits are primarily pain management and appetite stimulation, while the psychoactive components of cannabis have been used recreationally. Following the illegalization of other drugs like opioids in the 1800–1900's cannabis became a banned, or controlled, substance in many regions of the world. Nevertheless, the diverse medicinal properties of cannabis, like the seeds being used as a laxative, meant its use prevailed in several countries (Zuardi, 2006; Ashton, 2001; Baron, 2018; Borgelt et al., 2013; Walsh et al., 2013). In recent years, many regions and nations have decriminalized or legalized cannabis use. Notably, cannabis use became legal in Canada in October 2018.

Increasing legalization and decriminalization of cannabis has dramatically increased recreational use over the last decade. While global numbers of cannabis users have remained stable, from 2007 to 2017 the number of recurring-

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users increased by about 70% in the United States and daily users more than doubled (UNODC, 2017; UNODC, 2019. In 2018, it was estimated that there were 188 million global cannabis users, placing it as the most widely used drug in the world (UNODC, 2017). Numerous reports have indicated high prevalence of pesticides on illegal crops or medicinal crops, leading to strict requirements for quality assurance and quality control (QA/QC) of cannabis products to ensure the health and safety of consumers (Stone, 2014; Dryburgh et al., 2018; Moulins et al., 2018). New regulations in Canada stipulate that all active ingredients and contaminants present in the final product need to be reported and must be below maximum allowable levels for product release. Regulated contaminants of concern include pesticides, heavy metals, microbials, and in some regions, residual solvents (APHL Guidance, 2016; Government of Canada Law, 2018; Craven et al., 2019; Tran et al., 2018) . Health Canada has released a watch list of 96 pesticides with a maximum allowable level in the range of 0.01 – 1 μg/g for each class of cannabis product: fresh plant, dried plant, or oil (Government of Canada Law, 2018; Craven et al., 2019). However, because of the speed at which legalization occurred the released regulatory values are incomplete, as a few pesticides are missing some, or all, of the maximum allowable level for each product type (denoted by asterisks in SI Table S8) (Government of Canada Law, 2018; Craven et al., 2019).

Similar to the control of pesticides in food products, the control of pesticides in production of cannabis products is regulated under the Pest Control Act of Canada (Government of Canada Law, Pest Control Products Act S.C. 2002, c. 28). All materials used from seeds to the final products must be certified and documented. The strict QA/QC for pesticide residues is necessary because of their potential adverse health effects (Tran et al., 2018; EN 15662 Method; Barcelo, 1993; Damalas and Eleftherohorinos, 2011). These include carcinogenicity, mutagenicity, and other toxic effects, some of which may be lethal at sufficient exposure (Stone, 2014; Barcelo, 1993; Damalas and Eleftherohorinos, 2011; Mostafalou and Abdollahi, 2017; Derbalah et al., 2019; Fu et al., 2018; Mostafalou and Abdollahi, 2013; Ye et al., 2017). Additionally, some pesticides are persistent pollutants and can remain in the environment long-term (Damalas and Eleftherohorinos, 2011; Arias-Estevez et al., 2008; Peterson et al., 2017). While the requirements for cannabis products are clear, there are no regulations or guidelines for acute or chronic occupational exposure within the growing facilities. Research on the presence of pesticides in cannabis growing facilities and growers' exposure to pesticides is scarce. Therefore, we investigated the occurrence of pesticides in one certified and one non-certified cannabis growing facility.

To assess pesticide occurrence in cannabis growing facilities, we developed a new high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) method for the analysis of 82 of the 96 pesticides listed by Health Canada. We also implemented a simple sampling method for monitoring pesticides in these facilities. The wipe sampling procedure was used to investigate the presence of pesticides. The non-certified site has protocols and guidelines still under review and was awaiting their license at the time of sampling. The certified facility has a license to grow cannabis and has established proper protocols and guidelines for all workers.

1. Experimental section

1.1. *Chemicals and materials*

Formic acid (FA) and syringe filters (PVDF, 0.22 μm) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Optima water, methanol (MeOH), acetonitrile (ACN), and pesticide standards (listed in Appendix A Table S1) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). The HPLC column was purchased from Restek (Bellefonte, PA, USA). Internal standards were ordered from Toronto Research Chemicals (TRC; North York, ON, USA). Polypropylene centrifuge tubes were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

1.2. *HPLC-MS/MS analysis of pesticides*

The HPLC-MS/MS method was first developed and validated with standards in our laboratory at the University of Alberta using a Shimadzu (Kyoto, Japan) HPLC system with a 5500 Sciex MS system (Sciex, Concord, ON, USA). A Restek biphenyl column (100 mm x 3.0 mm, 4.5 μm particle size; Bellefonte, PA, USA) was used for the separation and kept at 40 °C. The mobile phase included solvent A consisting of water containing 0.1% FA and 5 mmol/L ammonium formate, and solvent B containing MeOH with 0.1% FA and 5% water (mobile phases prepared V/V). The mobile phase was at a flow rate of 0.8 mL/min. The HPLC system was equipped with an autosampler and the injection volume was 3 μL. The optimized gradient elution was detailed in Appendix A Tables S2 and S3. The optimized method was used to determine LOD and extraction efficiency from the kimwipes in the University of Alberta laboratory.

The validated method was transferred to the Molecular Science Co (MSC) mobile laboratory equipped with a Shimadzu (Kyoto, Japan) HPLC system with a 6500+ Sciex MS system (Sciex, Concord, ON, USA). The same type of column, Restek biphenyl column (100 mm x 3.0 mm, 4.5 um particle size; Bellefonte, PA, USA), and the same composition of the mobile phase was used for the analysis. Injection volume was 3 μL. To increase the sample throughput, we increased the flow rate to 1.0 mL/min and the column temperature to 50 °C. The gradient program was re-optimized and is described in Appendix A Tables S4 and S5. The method was re-validated by analysis of the standards to provide consistent results with those at the university laboratory. All samples collected from the non-certified and certified site were analyzed using the HPLC-MS/MS (Sciex 6500+) method in the MSC mobile laboratory.

Multiple reaction monitoring (MRM) mode was used to detect and quantitate pesticides and internal standards for both methods. The MRM transitions for individual pesticides and the selected internal standards were presented Appendix A Table S1. Confirmation and identification of pesticides in the samples were achieved based on the match of retention time, detection of both MRM transitions, and their ion ratios to those of the commercial standards. Detailed information about the method is available in the Appendix A.

1.3. Control experiments for wipe sampling tests

First, two types of sampling surfaces were tested for extraction: a polypropylene and a glass surface, each approximately 930 cm^2. This was to evaluate feasibility of the wipe sampling. Each surface was washed four times, with 5 mL each of MeOH followed by ACN, water, and finally MeOH. Surfaces were left to dry fully after each wash. 1 mL of a 500 µg/L pesticide mix was deposited onto the surface. In total, there were six surfaces tested for recovery, 3 plastic and 3 glass. After drying, a Kimwipe (21 × 11 cm) was used to thoroughly wipe along the surface. The wipe was then placed into a new clean tube (that is sterile 50 mL-polypropylene centrifuge tube). A volume of 10 mL of MeOH was added into the tube to extract the pesticides on the wipe. The tubes were sonicated (in a water bath) for 10 min. Liquid samples were collected from the tubes and filtered through a 0.22 µm PVDF 13 mm diameter filter unit (Merck Millipore, Burlington, MA, USA). The procedures of the extraction are described in Appendix A Fig. S1.

1.4. Sample collection

Samples were collected from two different growing sites in British Columbia, Canada, where we got permission to access through the collaboration with Molecular Science Co. These sites will be referred to as non-certified and certified. Samples were taken from common surfaces, though the certified site had more extensive sampling done because of longer time for the access. Common surfaces included door handles, light switches, plant pots, metal stems for plant containment, and other similar surfaces. The non-certified site had additional swabs taken from the water sprayer nozzle and the water reservoir. The certified site involved a greater number of individual swabs of different apparatus such as the HVAC screen, dry room screen, twister trimmer, dry room, solvent wash, doors, and handles.

Inequal access to the two growing facilities resulted in differences in the number of samples and sample types obtained. As many surfaces were sampled as possible within the limited facility access and sampling time constraints at the non-certified site. At the certified site, permission was granted to collect samples without time restriction, therefore samples covered the entire facility. At both sites, all of the surfaces were sampled with a wipe, then stored in new, clean sterile 50 mL-polypropylene centrifuge tubes. A schematic of the sampling procedure is described in Appendix A Fig. S1. The area wiped was estimated for the swabs and values are reported in µg of pesticide/square centimeter of area wiped (Appendix A Table S7).

1.5. Extraction

Stored wipes were extracted using the same liquid extraction method described in Section 1.3. To extract pesticides, 10 mL of MeOH was added to each of the 50 mL-polypropylene tubes and spiked with the internal standards each at 10 µg/L final concentration. The sample vials were thoroughly shaken (by hand) for 5 min. Following, samples were filtered through a 0.22 µm PVDF 13 mm diameter filter unit. Additional details are provided in Appendix A Fig. S1.

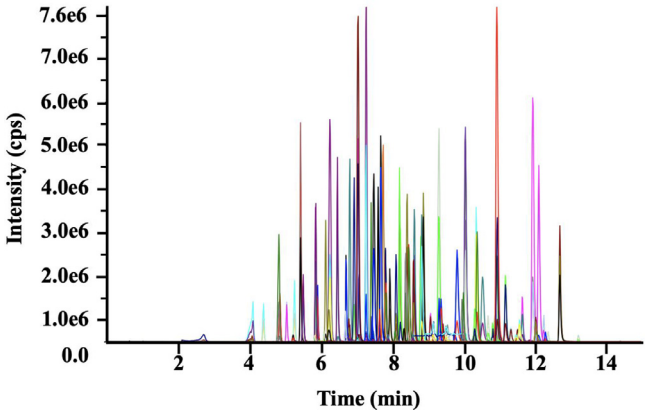

Fig. 1 – HPLC-MS/MS chromatogram of the 82 out of the 96 standard pesticide mix. Gradient separation performed with a Restek biphenyl column at 40 °C at the university laboratory.

2. Results and discussion

2.1. HPLC-MS/MS method

We have successfully developed a novel, accurate, sensitive, and fast HPLC-MS/MS method for the detection and quantitation of 82 of the 96 Health Canada pesticides using an optimized and scheduled MRM approach. Fig. 1 shows the separation of the pesticide standards at 50 µg/L. This method achieves an instrument limit of detection (LOD at S/N = 3) in the range of 0.02–5 µg/L for all but six pesticides. Methyl parathion, permethrin, cypermethrin, MGK-264, azadirachin, and daminozide, had LODs (at S/N = 3) of 6, 9, 15, 20, 37, and 93 µg/L, respectively (Appendix A Table S8).

Of the remaining 14 pesticides on Health Canada's watch list, seven were detectable but not quantifiable using the LC-ESI-MS/MS method. The seven non-detectable pesticides were abamectin, endosulfan (alpha and beta) sulfate, etridiazole, fenvalerate, kenoprene, phenothrin, and quintozene (also known as pentachloronitrobenzene). These seven pesticides are not readily compatible with LC or ESI-MS, so they are not detected. However, other methods, including APCI-MS or GC-MS, have detected these seven pesticides (Craven et al., 2019; Alder et al., 2006; Dalmia et al., 2018). Next, we further demonstrated the application of our method for environmental studies by screening two cannabis growing facilities that we got permission to access.

2.2. Swiping sampling and extraction

We investigated the occurrence of pesticides in two cannabis growing facilities because no study has yet to report on this. To rapidly screen the facilities, we used a modified wipe sampling method based on the United States Pharmacopeia (USP) method for inspecting contamination of hazardous drugs in healthcare settings (USP, 2017). Appendix A Fig. S1 shows the procedures of sampling and extraction schematically.

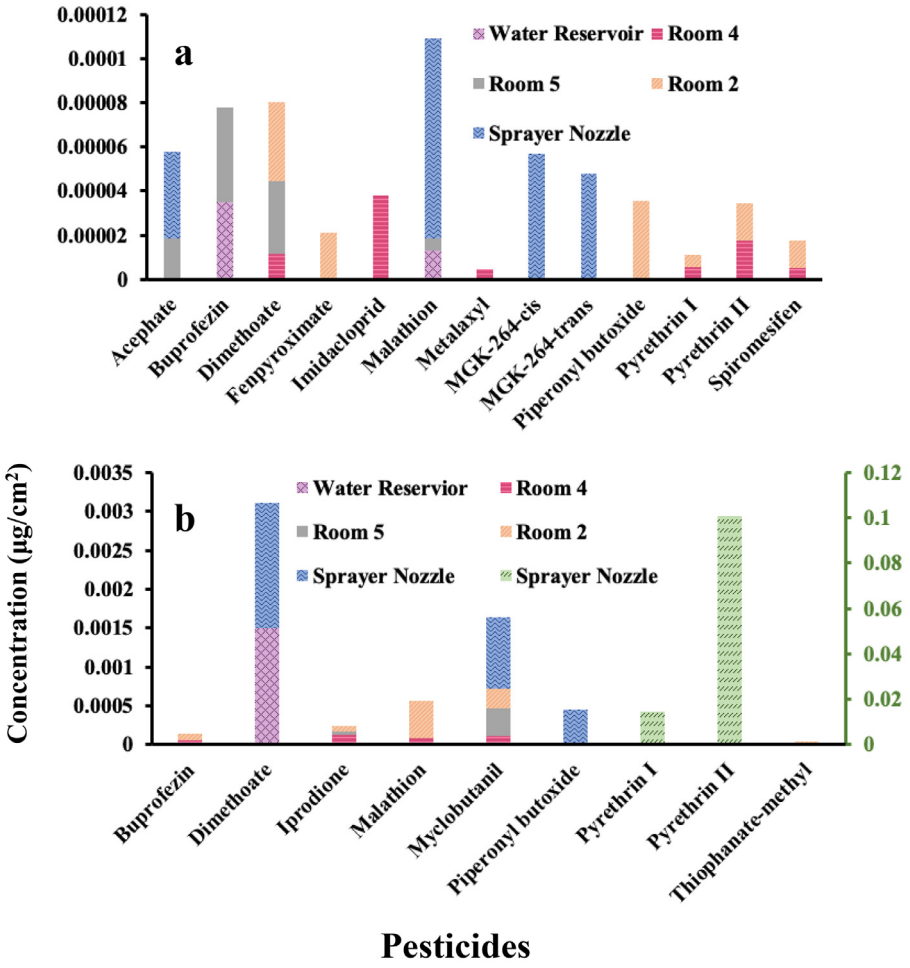

Fig. 2 – Estimation of the amount of pesticide per square centimeter detected at the non-certified growing facility. (a) Shows the mid-range of pesticides from $2.8e^{-5}$–$9.0e^{-5}$ $\mu g/cm^2$. (b) Shows the highest range of pesticides detected, detected greater than $9.0e^{-5}$ $\mu g/cm^2$. The secondary y-axis represents pyrethrin I and pyrethrin II, which were detected at higher concentrations than the other pesticides.

The sampling method was designed to be simple and sufficient for qualitatively inspecting facilities, as the USP method intended. As shown in Appendix A Fig. S4, the estimated average recoveries from the simulated sampling method ranged from 1% to 126% on glass and plastic surfaces because of the vast differences in the physicochemical properties of the 96 pesticides. Further details on the simulated sampling method is described in Appendix A Fig. S4 and Table S9.

2.3. *Pesticides in two growing facilities*

Two cannabis growing facilities at different stages of licensing, non-certified and certified, were sampled with the wipe sampling method. Sampling at each location was dictated by the level of access provided by the facility, and each wipe was extracted in 10 mL MeOH. Fig. 2 shows the estimated amount of pesticides per square centimeter of area wiped at the non-certified site: 13 pesticides at $2.8e^{-5}$ – $9.0e^{-5}$ μg/cm^2 (Fig. 2a) and 9 pesticides greater than $9.0e^{-5}$ μg/cm^2 (Fig. 2b). Additional data for the pesticides detected at less than $2.8e^{-5}$ μg/cm^2 are provided as Appendix A Figs. S2 and S3. In the 10 mL extracts, Pyrethrin I and II were estimated at 0.01 and 0.1 μg/cm^2, respectively, which were orders of magnitude higher than any of the other detected pesticides.

Fig. 3 shows the estimated amount of pesticides per square centimeter detected throughout the certified site. A total of 41 pesticides were detected at the non-certified site. This is in stark contrast to the certified site, where a total of 6 pesticides were detected, all at estimated amounts below 0.0000002 μg/cm^2. Although we only received access to screen two sites, the results highlight the occurrence of pesticides in growing facilities and the workers' exposure. Therefore, it is needed to regularly screen the facilities to eliminate pesticides. The results indicate the need to screen the materials (e.g. seeds, soil, water, and nutrients) used to grow cannabis. It is necessary to establish strict protocols for QA/QC and personal safety throughout the facility, supported by the fact that the certified site has significantly less pesticides detected. For example, proper personal protective equipment, watering and spraying, and thorough cleaning of various apparatus as well as the growing rooms was enforced and regularly performed. The non-certified site had incomplete or fewer protocols in

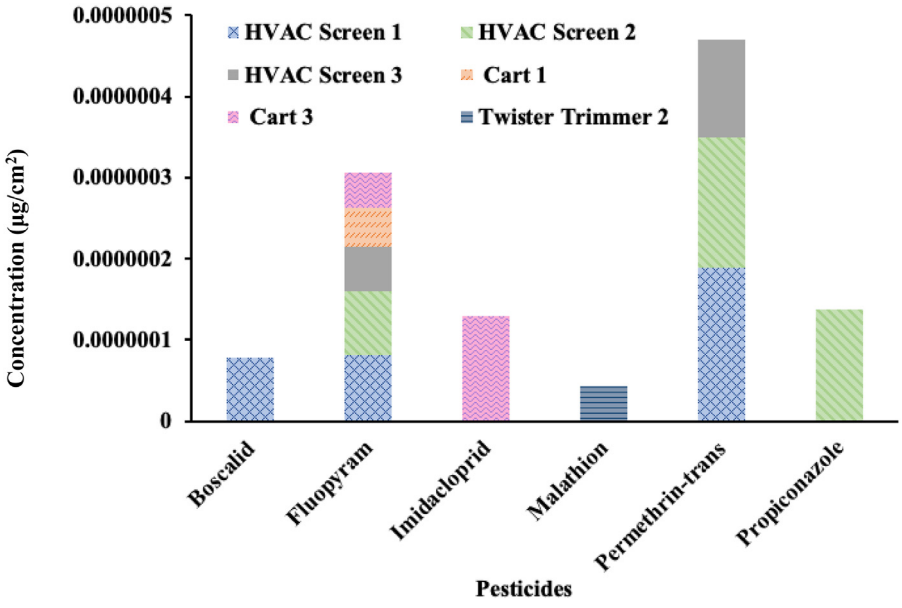

Fig. 3 – Estimation of the amount of pesticide per square centimeter detected at the certified growing facility.

place at the time of sampling. The simple wipe test developed for this study can help growers improve their protocols to ensure safe working conditions for workers in the facilities as well as better QA/QC for cannabis products. In fact, follow-up communication with the non-certified growing facility regarding these results helped the site to actively clean up and improve the protocols within the facility.

3. Perspective

The results of this study highlight the need for proper monitoring of pesticides in cannabis growing facilities, not just in the final consumer product. The simple wipe sampling procedure with HPLC-MS/MS analysis demonstrated the occurrence of these pesticides at two different cannabis growing facilities. This is the first study of its kind to highlight the need of routine monitoring of these pesticides in farming facilities to eliminate the pesticides in final products, as well as to reduce workers' occupational exposure. The routine screening will lead to a clean product, which is important to the financial outcome of the producers and health safety of the consumers and workers. The screening method is also useful for monitoring pesticides in other produce and food production facilities.

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Appendix A Supplementary data

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jes.2020.11.004.

REFERENCES

Alder, L., Greulich, K., Kempe, G., Vieth, B., 2006. Residue analysis of 500 high priority pesticides: better by GC-MS or LC-MS/MS? Mass Spectrom. Rev. 25 (6), 838–865.

Andre, C.M., Hausman, J.F., Guerriero, G., 2016. Cannabis sativa: the plant of the thousand and one molecules. Front. Plant Sci. 7, 17.

Arias-Estevez, M., Lopez-Periago, E., Martinez-Carballo, E., Simal-Gandara, J., Mejuto, J.C., Garcia-Rio, L., 2008. The mobility and degradation of pesticides in soils and the pollution of groundwater resources. Agric. Ecosyst. Environ. 123 (4), 247–260.

Ashton, C.H., 2001. Pharmacology and effects of cannabis: a brief review. Br. J. Psychiatry 178, 101–106.

Association of Public Health Laboratories (APHL), Guidance for state medical cannabis testing (2016). https://www.aphl.org/aboutAPHL/publications/Documents/EH-Guide-State-Med-Cannabis-052016.pdf (accessed September 30, 2020).

Baron, E.P., 2018. Medicinal properties of cannabinoids, terpenes, and flavonoids in cannabis, and benefits in migraine, headache, and pain: an update on current evidence and cannabis science. Headache 58 (7), 1139–1186.

Barcelo, D., 1993. Environmental Protection Agency and other methods for the determination of priority pesticides and their transformation products in water. J. Chromatogr. 643 (1–2), 117–143.

Borgelt, L.M., Franson, K.L., Nussbaum, A.M., Wang, G.S., 2013. The Pharmacologic and clinical effects of medical cannabis. Pharmacotherapy 33 (2), 195–209.

Craven, C.B., Wawryk, N., Jiang, P., Liu, Z., Li, X.-F., 2019. Pesticides and trace elements in cannabis: analytical and environmental challenges and opportunities. J. Environ. Sci. 85, 82–93.
Dalmia, A., Cudjoe, E., Astill, T., Jalali, J., Weisenseel, J.P., Qin, F., et al., 2018. LC-MS/MS with ESI and APCI sources for meeting California cannabis pesticide and mycotoxin residue regulatory requirements. Cannabis Sci. Technol. 1 (3), 7.
Damalas, C.A., Eleftherohorinos, I.G., 2011. Pesticide exposure, safety issues, and risk assessment indicators. Int. J. Environ. Res. Public Health 8 (5), 1402–1419.
Derbalah, A., Chidya, R., Jadoon, W., Sakugawa, H., 2019. Temporal trends in organophosphorus pesticides use and concentrations in river water in Japan, and risk assessment. J. Environ. Sci. 79, 135–152.
Dryburgh, L.M., Bolan, N.S., Grof, C.P.L., Galettis, P., Schneider, J., Lucas, C.J., et al., 2018. Cannabis contaminants: sources, distribution, human toxicity and pharmacologic effects. Br. J. Clin. Pharmacol. 84 (11), 2468–2476.
Fu, L., Lu, X.B., Tan, J., Wang, L.X., Chen, J.P., 2018. Multiresidue determination and potential risks of emerging pesticides in aquatic products from Northeast China by LC-MS/MS. J. Environ. Sci. 63, 116–125.
Government of Canada Law, Canada Justice Cannabis Regulations 2018 (Registration 2018-06-27). https://laws-lois.justice.gc.ca/eng/regulations/SOR-2018-144/page-1.html#fn_81200-2-417E_hc_19057-ID0EOFAA (accessed September 30, 2020).
Governement of Canada Law, Pest Control Products Act S.C. 2002, c. 28 (https://laws-lois.justice.gc.ca/eng/acts/p-9.01/page-1.html (Accessed September 30, 2020)
Mercuri, A.M., Accorsi, C.A., Mazzanti, M.B., 2002. The long history of cannabis and its cultivation by the Romans in central Italy, shown by pollen records from Lago Albano and Lago di Nemi. Veg. Hist. Archaeobot. 11 (4), 263–276.
Mostafalou, S., Abdollahi, M., 2017. Pesticides: an update of human exposure and toxicity. Arch. Toxicol. 91 (2), 549–599.
Mostafalou, S., Abdollahi, M., 2013. Pesticides and human chronic diseases: evidences, mechanisms, and perspectives. Toxicol. Appl. Pharmacol. 268 (2), 157–177.
Moulins, J.R., Blais, M., Montsion, K., Tully, J., Mohan, W., Gagnon, M., et al., 2018. Multiresidue method of analysis of pesticides in medical cannabis. J. AOAC Int. 101 (6), 1948–1960.
Peterson, E.M., Wooten, K.J., Subbiah, S., Anderson, T.A., Longing, S., Smith, P.N., 2017. Agrochemical mixtures detected on wildflowers near cattle feed yards. Environ. Sci. Technol. Lett. 4 (6), 216–220.
Stone, D., 2014. Cannabis, pesticides and conflicting laws: the dilemma for legalized States and implications for public health. Regul. Toxicol. Pharmacol. 69 (3), 284–288.
Touw, M., 2018. The religious and medicinal uses of cannabis in China, India and Tibet. J. Psychoact. Drugs 13 (1), 23–34.
Tran, K., Twohig, M., Young, M., Aubin, A., Meruva, N., Fujimoto, G., et al., 2018. Determination of the oregon pesticide list in cannabis using a simple extraction procedure with DSPE cleanup and UPLC-MS/MS. Waters 2018 Application Note (1–7), http://www.waters.com/webassets/cms/library/docs/720006373en.pdf (accessed September 30, 2020).
European EN 15662 method, 2018. Foods of plant origin — Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and cleanup by dispersive SPE — QuEChERS-method. https://infostore.saiglobal.com/en-us/Standards/EN-15662-2018-326900_SAIG_CEN_CEN_753112/
UNODC (United Nations Office on Drug and Crime), World Drug Report 2017. https://www.unodc.org/wdr2017/field/Booklet_1_EXSUM.pdf (accessed September 30, 2020).
UNODC (United Nations Office on Drug and Crime), World Drug Report 2019. publication, U. N., Ed. 2019; p. 78. https://wdr.unodc.org/wdr2019/prelaunch/WDR19_Booklet_1_EXECUTIVE_SUMMARY.pdf (accessed September 30, 2020).
USP, USP General Chapter <800>Hazardous Drugs - Handling in Healthcare Settings. 2017. https://www.usp.org/sites/default/files/usp/document/our-work/healthcare-quality-safety/general-chapter-800.pdf (accessed September 30, 2020)
Walsh, Z., Callaway, R., Belle-Isle, L., Capler, R., Kay, R., Lucas, P., et al., 2013. Cannabis for therapeutic purposes: patient characteristics, access, and reasons for use. Int. J. Drug Policy 24 (6), 511–516.
Ye, M., Beach, J., Martin, J.W., Senthilselvan, A., 2017. Pesticide exposures and respiratory health in general populations. J. Environ. Sci. 51, 361–370.
Zuardi, A.W., 2006. History of cannabis as a medicine: a review. Rev. Bras. Psiquiatr. 28 (2), 153–157.