Pesticides in cannabis: A review of analytical and toxicological considerations

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Abstract

A review of the literature surrounding the use, analysis and detection of pesticide material for cannabis cultivation is presented. The use of pesticides in crop cultivation is not new, and cannabis crops are no exception. Studies have found that the use of these are common and that high levels of the pesticides are transferred into the cannabis smoke.

The most common pesticides classes associated with cannabis are insecticides, acaricides and fungicides. Over 350 different pesticide products may be used on cannabis materials and of these, 16 pesticides and 3 plant growth regulators (PGR) are considered to be the main candidates. Many of the pesticides found in cannabis samples destined for consumption are classed as moderately hazardous by the World Health Organisation. Analytical methods for pesticide detection on cannabis are being developed with a view to implement quality control to cannabis where it is legal before being sold. However, no standardised protocol exists. The pesticide levels found in the cannabis samples tested were generally low (less than $\mu g \cdot g^{-1}$), these results do not however provide information on chronic low-dose adverse effects of pesticides in relation to cannabis consumption. Currently no research exists on the toxicity of pyrolysed pesticides in humans from smoking cannabis. More studies are needed to further understand this potentially harmful health threat.

1. Introduction

Cannabis is the most widely used drug globally. Between 2010 and 2016, 145 countries reported growing cannabis on a regular basis. Smoking herbal cannabis is the most common method of administering this drug. Cannabis is currently a class B controlled substance in the UK [1]. The UK government was reluctant to legalise this drug for medical use until recently. Expert doctors have been given permission to issue prescriptions for cannabis-based medicines since 1st November 2018 [2]. Ironically, the UK produced 45% of the total legal cannabis destined for medical use in 2016 [3]. In the US, cannabis is federally prohibited for any use under the Controlled Substances Act (CSA) 1970. However, it is legal for medicinal and/or recreational purposes in 50% of the states. This causes much confusion among patients and healthcare providers [4]. This plant has acquired substantial attention in recent years as an increasing number of countries are legalising the drug for medicinal as well as recreational use [5].

Cannabis, similarly to other plants, is prone to diseases, pests, fungi, and bacterial infections. Due to its increasing popularity, especially in countries and states where it is now legal for both medicinal and recreational purposes, growers are more inclined to use plant growth stimulators and pesticides to increase and accelerate yield [6]. This issue is widely overlooked since cannabis is still illegal in most parts of the world. There is both a lack of monitoring for the use of pesticides on legal cannabis plantations as well as a lack of knowledge on how pesticides that have undergone pyrolysis in cannabis affect the smoker. Few studies bridge this gap between detected residue levels and internal doses of pesticides resulting from usage of cannabis products [7]. There are currently no accredited or verified methods to test cannabis for pesticides and very little data is published on this topic [8].

This review aims to assess current data with respect to pesticides used on cannabis materials. Analytical procedures that can detect these chemicals within a cannabis sample are documented and evaluated. Finally, toxicological implications of smoking pesticide - laced cannabis are discussed.

2. Pesticides

Pesticides are classed into seven major groups: insecticides, herbicides, fungicides, rodenticides, acaricides, molluscicides and nematocides. This classification is based on the field of use, however, they can also be classified by chemical class. There are organophosphorus compounds (OP), carbamates, chlorinated hydrocarbons, pyrethroids and heterocyclic compounds. Figure 1 shows examples of the variable structures of selected pesticides based on their classification.

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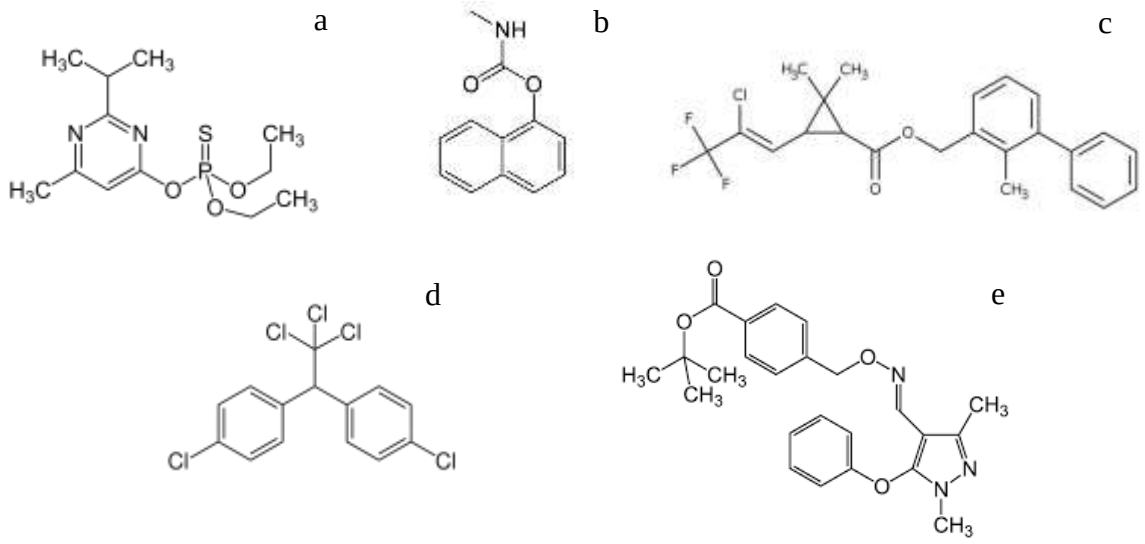

Figure 1 Pesticides from different chemical classes, showing pesticide variability. a: Diazinon (OP), b: Carbaryl (carbamate), c: Bifenthrin (pyrethroid), d: DDT (chlorinated hydrocarbon/organochlorine), e: Fenpyroximate (heterocyclic compound)

Most of these can be used on a variety of different targets. Commercial formulations may even mix compounds from these different classes into one product [9]. Numerous pesticides are notorious for their negative health impact on humans and the environment, resulting in their restricted use or a total ban. Pesticides acute toxic effects following a high dose oral exposure are well documented. Although pesticides may not pose an immediate threat to consumers in small quantities, chronic low-dose adverse effects could be substantial, yet knowledge on this subject is limited [10]. Since cannabis is mainly smoked, the guidelines and reported toxic levels of pesticides risk being inexact, as these levels are associated with agricultural products that are destined to be orally ingested [11]. It has been reported by the Cannabis Safety Institute "that pesticide residue on retail cannabis products is often found at levels exceeding the allowable levels on any agricultural product" [12]. Moreover, it is reported that the metabolites of many pesticides are more toxic than their parent compounds [9]. A study by Dryburgh et al., highlighted pesticide metabolites will more than likely be present when cannabis is smoked [13]. Most pesticides target the central nervous system and thus could affect more than just the intended pest posing a health threat to humans and other animals [14]. Pesticides may present a danger to users with epilepsy and other neurological conditions by binding to certain receptors in the brain. This could pose a significant threat to medicinal cannabis users who already have negative health complications [15].

2.1 Pesticides Associated with Cannabis

The most common pests associated with indoor cannabis flowers and leaves are aphids, spider mites and thrips [16]. Fungal diseases are also problematic in greenhouses and when using indoor lighting systems. Consequently, the most common pesticides associated with cannabis are insecticides, acaricides and fungicides [17]. Cannabis is an illegal drug in most countries, therefore there are no guidelines for pesticide use on cannabis cultivation. In the countries where it is legal, whether that be for medicinal or recreational use, the originality of the situation makes it so that no guidelines currently exist [18]. Moreover, many legislations do not prioritise this issue in routine analysis of cannabis samples. A study found that 44% of 1722 growers in Australia, Denmark and the UK admit to using chemical fertilisers, supplements or insecticides [19]. This is a large number, taking into account that some growers may not have taken part in the survey due to fear of being discovered. A recent study examining legalised cannabis products in Washington State found that 84.6% of the samples contained significant quantities of pesticide residues, although no concentrations were mentioned [20]. Medical cannabis products tested in California

had pesticides in 49.3% of the samples [21]. In the US, it is illegal to use a pesticide on a crop that it is not specifically designed for [22]. The Environmental Protection Agency (EPA) claims: “We have yet to receive any applications for pesticide use on marijuana and therefore, have not evaluated the safety of any pesticide on marijuana” [23]. Furthermore, setting tolerance limits is complex and time-consuming. In the absence of federal regulations, states have individually formulated guidelines [24].

Washington State Department of Agriculture (WSDA) has recently released a list of 271 pesticide products approved for use on cannabis [17]. The state of Colorado has compiled a list of 357 pesticide products, many of which contain the same active ingredients, that are legal to use on cannabis plantations [24]. Historically, some of these pesticides have been approved for use on landscape plants, not plants destined for consumption [25].

It is believed that indoor plants are at higher risk of pesticide contamination as opposed to plants grown outdoors [26]. Most cannabis plants are now grown indoors to ensure year round production and consistent THC levels. Moreover, pesticide concentrations in full-grown plants are higher than those found in younger plants due to receiving more pesticide sprayings and pesticides accumulating in the plant [27].

Table 1 displays data on pesticides found in cannabis samples from different countries. In one study, the levels of tebuconazole and bifenthrin were found to be within the LD_{50} (median lethal dose) range [28]. Bifenthrin is a pyrethroid insecticide and classed as moderately hazardous by the World Health Organisation (WHO). It is said to have relatively low toxicity to humans, however, when inhaled it can cause localised reactions in the respiratory tract such as shortness of breath, chest pain, coughing and oedemas. Some may experience an asthma-like attack that could prove fatal [9]. Tebuconazole is a triazole fungicide and classed as moderately hazardous by the WHO. Oral exposure has low toxicity, although when inhaled it can cause nose, lung and throat irritation. The Enviromental Protection Agency (EPA) have classed it as a possible human carcinogen after observing liver tumors in mice when exposed orally to high doses of this pesticide [29]. Another study conducted in Belgium detected 19 pesticides including Dicrotophos, Chlorfenvinphos and Dichlorvos. Additionally, Dicrotophos and Dichlorvos are not approved for use in the EU [27].

As seen in Table 1, the levels of pesticides found in the different studies vary largely. Many factors could influence the results such as pesticide dosage and application frequency. The herbal material

analysed in these studies reported herein were seized by the police. It is unknown how the growers treated the plants [27]. Moreover, some studies do not actually give the quantity of pesticides found, only that they were detected. The pesticides found were mainly low and on the lower end of the World Health Organisation's (WHO) toxicity classification. However, it must be stressed that these levels were found on unsmoked cannabis and the WHO toxicity levels are associated with oral toxicity in rats. These results should encourage future monitoring of pesticide residues in legal cannabis productions to evaluate safety for consumers.

2.2 Pyrolysis of Pesticides in Cannabis

When smoking cannabis with a cotton filter, users may be exposed to up to 30.1% of the pesticide residues from the original plant, whereas without a filter this could be as high as 69.5% [30]. Cannabis is typically smoked without a filter, therefore exposing the user to higher levels of dangerous compounds and their degradation products. Cannabis smoke contains many of the same carcinogens as tobacco smoke and reportedly four times as much tar as tobacco [31]. When inhaling smoke, it directly reaches the blood stream through the lung gas exchange, making bioavailability much higher than if the product was to be ingested for example [32]. Additionally, cannabis smokers inhale two-thirds more per "puff" and retain the inhalation for longer than if they were to smoke a cigarette [33]. When smoked with a water pipe filter, the levels of pesticides found in the cannabis smoke (0.08-10.9% recovery) were similar to the levels found in tobacco smoke (2-16% recovery) [30]. However, this study only addressed how much of the pesticide residues might reach the lungs, not the adverse effects that these levels could have on the user. This study also only used spiked material. A mixture of varying concentrations of pesticides were added to the leaf material. This method was optimised for the study of pesticides in smoke, although, it may not be not representative of what might be found in real samples, as growers' application methods and quantities are unknown [27].

Pesticide residues in cannabis will undergo pyrolysis and will be inhaled when smoking. Although it is yet unknown how harmful these chemicals are to humans when pyrolysed and inhaled, the fact that up to 69.5 % of residues are present in cannabis smoke should be of concern [34]. Moreover, a study looking at cannabis smoke alone found that it contained 20 times more ammonia than tobacco smoke and 3 to 5 times more hydrogen cyanide, nitric oxide and aromatic amines than in tobacco [35]. The levels of toxicity while smoking cannabis containing pesticide residues could be compared to those of tobacco containing pesticide residues [28]. It has been shown that

pyrothroid insecticides on tobacco transfer into cigarette smoke. However, most of the residues were found within the cigarette's cotton filter [36]. Furthermore, there could be different pharmacokinetic interactions between the cannabinoids and the contaminants. For example, it has been shown that THC may have protective properties against the other harmful components found in cannabis smoke [31].

Pyrolysis may transform some pesticides into more toxic forms that are then inhaled [13]. For example, the fungicide myclobutanil, has been found in cannabis samples in a number of studies and is known to decompose into hydrogen cyanide when heated [6, 8, 12, 20, 27, 37]. Hydrogen cyanide causes neurological, respiratory, cardiovascular and thyroid problems and can also be lethal [38]. The World Health Organisation have classified it as moderately hazardous. Myclobutanil has been banned for use on cannabis in Oregon, but is allowed in Nevada at limited quantities [24]. Currently, there is a paucity of data concerning how other pesticides react when heated and what acute and chronic effects these would have on the user. More studies on cannabis smoke need to be conducted to better understand these effects. Currently, there are no reported cases in the literature of intoxication from pesticides or its metabolites due to smoking cannabis. However, there are several case reports of patients experiencing aspergillosis associated with smoking cannabis [39].

3. Sampling and Analysis

3.1 Cannabis analytical methods

The main rationale for testing cannabis analytically is to confirm its identity as well as test its potency. When analysing cannabis, total THC content is of most interest because this gives a potency indication [40]. Prior to instrumental analysis, herbal cannabis is preferably dried, pulverised and sieved to ensure homogeneity. Gas Chromatography (GC) and Liquid Chromatography (LC) coupled with Mass Spectrometry (MS) are the most common methods for analysing cannabis and a broad range of cannabinoids. Best sensitivity is generally obtained through GC-MS or GC-FID (flame ionisation detector), using low polarity stationary phases, as the main functional units of cannabinoids are phenolic. These are the recommended methods by the United Nations for the analysis of cannabis and cannabis products [40]. Although, samples

may need to be derivatised as the carboxylic acids degrade when placed into the injection port of the GC instrument. Silylation is commonly used as it imparts more volatility to the cannabinoids [5]. As cannabis is mainly tested for THC and other cannabinoids, potential harmful compounds are generally overlooked.

3.2 Pesticide Analytical Methods

Over 700 pesticides are used routinely in the food industry and these foodstuffs are monitored for pesticide residues. GC-MS with electron impact (EI) or LC-MS/MS with electrospray ionisation (ESI) are the most common techniques applied to multi-residue analysis of pesticides [41]. Multi-residue methods facilitate efficient monitoring as they allow the detection of up to a few hundred compounds per extraction and per analytical run [42]. The chemical nature of the pesticides to be detected dictates whether a LC or a GC approach should be used. A study that tested 500 different pesticides found that LC-MS/MS with ESI provided better sensitivity for all classes of pesticides except for the organochlorine pesticides, which were best analysed by GC-MS [41]. LC-MS/MS is generally preferred over GC-MS as the tandem MS reduces matrix interferences. LC can also overcome issues associated with thermal instability. Another study used LC-MS for thermally labile and polar pesticides and GC-MS for volatile pesticides such as organophosphates and organochlorines [43]. The European Committee for Standardisation has developed a method for determining the levels of pesticide residues in foods of plant origin. It has been validated for the extraction of 80 pesticides belonging to various chemical classes [44]. This method uses GC-MS and LC-MS/MS following acetonitrile extraction and dispersive solid phase extraction (dSPE) QuEChERS (quick, effective, cheap, easy, rugged, safe) clean-up [45].

3.3 Pesticide Qualification and Quantification in Cannabis Samples

The American Herbal Pharmacopoeia (AHP) compiled a list of 16 pesticides and 3 plant growth regulators (PGR) that are most likely to be used on cannabis [25]. However, this is not relevant to every country as these may vary from region to region depending on product availability and on different regulations on pesticides in each country. Pesticides will only be present in small quantities in cannabis samples, in comparison to the larger THC levels that will be detected. This is because pesticide levels decline after application due to photo oxidation, volatilisation and biological degradation. The quantities found are generally in $\mu g \cdot g^{-1}$ or $ng \cdot g^{-1}$ [46]. Cuypers et al. (2013) claim that pesticides will accumulate in cannabis plants due to receiving multiple sprayings over their lifetime [27]. Results from table 1 show pesticide levels in the range of 0.01 to 800

$mg \cdot kg^{-1}$. The range in levels seen may be due in part to poor recovery with the analytical methods used. The percentage recoveries from some of the studies mentioned in table 1 ranged from less than 50% to more than 120%, with the majority of recoveries falling between 70 and 120% [6, 8, 28]. Another main issue is that pesticides are numerous and belong to a broad spectrum of chemical classes. Moreover, pesticides from completely different classes can be found in the same cannabis sample. They may be acidic, basic or neutral, varying in polarity and solubility. Some are thermally labile while others may easily bind onto surfaces [46].

Table 2 illustrates the analytical techniques used that successfully detected pesticides in cannabis. Not all studies quantified the pesticides found. It is important to note that these studies used a mixture of spiked material and seized plants. Therefore, some studies are looking at what truly is in samples whereas others are testing their analytical methods. The limits of quantification (LOQ) were only given for three of the studies that used LC-MS/MS. In a study conducted by Alder et al. in 2006, 500 pesticide residues were analysed by GC-MS and LC-MS/MS. It was determined that LC-MS/MS was a much more sensitive method due to the LOQs being much lower [41].

3.3.1 Sample Preparation

Cannabis is a highly complex matrix. Not only does it contain many cannabinoids but also many secondary cannabinoids and terpenoids. If pesticides are to be detected and quantified in cannabis samples accurately, a representative sample must be collected, homogenised, extracted with suitable solvents and interferences removed. The lower the target analyte concentration the more robust the clean-up needs to be [34].

Pesticides must be extracted from the sample matrix and isolated from any interfering materials for successful and accurate analysis. Cannabinoids are found in the glandular trichomes on the leaves and flowers which are highly resinous. Many pesticides are hydrophobic, therefore they adhere to these structures, making them hard to isolate from the matrix [46]. Moreover, cannabinoids and terpenes are hydrophobic and they are readily extractable by organic solvents, such as acetonitrile. This is why the European standard method (EN15662) uses citrate buffered QuEChERS as it does not co-extract the cannabinoids when trying to isolate the pesticides [8]. The EN15662 method is recommended for analysis of pesticides in foods of plant origin. It is not specifically recommended for pesticide analysis in cannabis, however, it has been modified and used for this purpose [8, 28]. This involves drying out the sample, coarsely grinding the herbal

material for homogenization, an acetonitrile extraction and QuEChERS dispersive SPE clean-up. The first step is the extraction which uses an organic solvent and salt solution (usually $MgSO_4$). The second step is dispersive SPE which further extracts and cleans the supernatant from the previous step. The purpose of this is to remove sugars, lipids, organic acids, sterols, proteins, pigments and excess water [47]. Lozano et al. [48] proposed using calcium chloride instead of magnesium sulphate (employed in the EN 15662 method) for the clean-up step as it reduced matrix effects and improved recovery, however this was only validated for tea samples.

The most universal extraction method is liquid-liquid extraction (LLE). However, this method is unsuitable for many analyses as is consumes a large amount of solvent as well as being a laborious method [49]. One study used headspace solid-phase micro extraction (SPME) of pesticides residues from cannabis samples. This method provides a simpler and more rapid quality control of plant material [49]. Unfortunately, this method is preferably coupled with GC-MS, which cannot analyse all types of pesticides [50].

EDGE® is a new system for extracting pesticides from cannabis. It includes a dispersive SPE clean-up phase in the same one sample cell. It offers the fastest automated pesticide extraction. It has been tested and has efficiently extracted over 400 pesticides from cannabis in under 7 minutes. Recoveries were between 80 and 115% for analysis with GC-MS and between 81 and 117% for analysis with LC-MS [51].

3.3.2 GC-MS

GC-MS is perceived as the reference method for identification and confirmation of pesticides in different materials. Libraries containing reference spectra for many pesticides as well as their metabolites and decomposition products are designed for use with GC-MS [9]. Sample preparation for GC-MS is usually SPME or QuEChERS. These samples can be directly injected in splitless mode and ionized using electron impact ionization (EI). GC-MS is a selective and sensitive method for volatile and hydrophobic pesticides such as organophosphates and organochlorines. However, GC-MS can be a source of pesticide artifacts and decomposition products due to light sensitivity, oxidation, hydrolysis and heat. Lack of volatility or thermal lability could be overcome with derivatisation, however it is not known in advance what a cannabis sample will contain and therefore is problematic to know which derivatising agent to use. Additionally, derivatising would only add to sample preparation time as well as complicating sample matrices [52]. This is why

many recent studies have used LC-MS/MS as it can reduce matrix interferences and overcome issues with thermally labile and highly polar pesticides as well as pesticides with high molecular weights [43].

3.3.3 LC-MS/MS

Historically, LC was used more rarely in the past because traditional detectors associated with this technique, such as UV, diode array and fluorescence were less selective and less sensitive than the detectors used with GC instruments [41]. However, in the last couple of decades, the development of atmospheric pressure ionization (APCI) and electron spray ionization (ESI) coupled with MS have largely increased the sensitivity of LC detection [41]. LC-MS/MS is preferred for ionic and polar pesticides. This is a much more versatile and universal technique compared to GC-MS, however, organochlorine pesticides are notoriously difficult to ionize with ESI [53]. Some chlorinated pesticides have been ionized with atmospheric pressure chemical ionization (APCI) and analysed with LC-MS to overcome the issues faced with ESI [52]. QuEChERS sample preparation can also be used for LC-MS/MS. However, this can be made more straight forward for analysis with LC-MS/MS by using LLE with acetonitrile as it requires fewer steps and is just as effective [52].

3.3.4 Other Methods

Supercritical fluid chromatography (SFC) offers high resolution. This method was used in a study that was able to rapidly and simultaneously detect pesticides over a wide range of polarities and molecular weights, including highly hydrophilic ones [54]. SFC eliminates the need for two different analytical instruments to analyse a single sample. The biggest drawback however, is that run times are short which may result in the presence of isobaric interferences, therefore reducing the method's resolution [52]. LC-UV has been successfully used for the quantification of paraquat in cannabis. This is an easy method to perform, however it is not suitable for multiresidue analysis. A study using capillary electrophoresis-UV detected three herbicides in cannabis [55]. This is a simple and fast separation method for highly polar pesticides that provides good resolution and recovery. Unfortunately, the LOQs (paraquat: 5 $\mu g \cdot g^{-1}$, glyphosate: 10 $\mu g \cdot g^{-1}$, AMPA: 10 $\mu g \cdot g^{-1}$) are deemed to be too high for the regulatory action limits [55]. For example, current LOQs for various pesticides in the state of Oregon lie between 0.00025 and 0.005 $\mu g \cdot g^{-1}$. Regulatory action limits lie between 0.1 and 2 $\mu g \cdot g^{-1}$. Similar values can be found for different states [56].

3.4 Towards Developing Standardised Testing

Due to the disparity of the legal status of cannabis worldwide, it is difficult to establish botanical and chemical quality standards for such materials [8]. Cannabis standards of common strains would be needed for efficient testing as the different properties from the different strains could alter results from pesticide analysis due to ion enhancement or ion suppression [57]. It cannot be ignored that standardised testing should be considered as becoming mandatory in countries where the drug is already legal. If implemented, this testing will also assist other countries that are planning on or in the process of legalising cannabis.

Due to the wide variety of physical properties of the pesticide components and the complexity of cannabis' matrix, developing a standardised method is proving complicated and involves numerous stages, particularly for sample preparation. Using both GC-MS and LC-MS provides complimentary analysis to cover the different polarity and thermal needs of the different pesticides. Integration of these two systems into a MS database would be extremely useful for laboratory testing as to cover a wide scope of pesticides [52]. However, these two analytical systems are expensive and analysis can be extensive due to instrument parameter modifications. Moreover, regulatory requirements for pesticide testing vary between countries and vary from state to state in the US, which in turn influences the sample preparation, instrumentation and techniques performed [53].

4. Summary

It is apparent that pesticide use on cannabis crops is ubiquitous. Appropriate residue levels on legal cannabis plantations need to be determined and quality controls implemented. Further research is required to assess how these pesticides affect the human body when pyrolysed as there is a paucity of data in this area. Knowing to what extent the consumer may inhale pesticide residues from smoking cannabis is also essential. To date there is only one comprehensive study conducted by Sullivan et al. [30] that aimed to bridge the gap between detected pesticide residue levels and internal pesticide doses. Further toxicity studies are therefore required to fully assess the fate and behaviour of pesticide materials on cannabis.

Numerous pesticides of different classes have been detected in cannabis materials. When quantification has been achieved, levels range from 0.01 to 800 ug g^{-1} although concentrations are generally found in the low ug g^{-1} region. The majority of these detected pesticides are classed as moderately hazardous. Analytical methods chiefly employed for this analysis are currently QuEChERS extraction with GC-MS or LC-MS/MS.

Pesticides determination in cannabis samples are not targeted with routine instrumental analysis. A few studies have successfully detected pesticide residues on cannabis. However, additional work is needed further develop rigorous methods that satisfy requirements for all analytes of interest. Development of these analytical techniques is challenging due to many factors such as cannabis matrix complexity, contaminants at trace-levels, simultaneous detection of multiple chemically variable contaminants and contrasting laws. In countries where medical and recreational cannabis is legalised, work towards pursuing a standardised protocol for pesticide detection, using standard cannabis reference materials should be considered.

References

[1] Misuse of Drugs Act (1971). Available at: https://www.legislation.gov.uk/ukpga/1971/38 (Accessed 28th January 2019).

[2] Stevens, A. (2018). Medical cannabis in the UK. *Br.Med.J.*, 363, pp.1-2.

[3] Hurley, R. (2018). Cannabis, cannabis everywhere: UK to review medical cannabis policy as Canada plans imminent legalisation for all uses. *Br.Med.J.*, 361, pp.1-3.

[4] Mead, A. (2017). The legal status of cannabis (marijuana) and cannabidiol (CBD) under U.S. law. *Epilepsy & Behavior*, 70, pp.288-291.

[5] Leghissa, A., Hildenbrand, Z. and Schug, K. (2017). A review of methods for the chemical characterization of cannabis natural products. *J. Sep. Sci.*, 41(1), pp.398-415.

[6] Dahl, J.H., Kowalski, J., Laine, D., Zitzer, J. (2016). Rapid Screening and Quantitation of Pesticide Residues in Cannabis by Modified QuEChERS and LC-MS-MS. *Shimadzu*. Available at: https://www.ssi.shimadzu.com/sites/ssi.shimadzu.com/files/Products/literature/lcms/LCMS-8050%20Application%20-%20TP-230_Pesticides%20Cannabis_Dahl_P.pdf (Accessed 28th January 2019).

[7] Leung, M., Silva, M., Palumbo, A., Lohstroh, P., Koshlukova, S. and DuTeaux, S. (2019). Adverse outcome pathway of developmental neurotoxicity resulting from prenatal exposures to cannabis contaminated with organophosphate pesticide residues. *Reprod. Toxicol.*, 85, pp.12-18.

[8] Pérez-Parada, A., Alonso, B., Rodríguez, C., Besil, N., Cesio, V., Diana, L., Burgueño, A., Bazzurro, P., Bojorge, A., Gerez, N. and Heinzen, H. (2016). Evaluation of Three Multiresidue Methods for the Determination of Pesticides in Marijuana (*Cannabis sativa* L.) with Liquid Chromatography-Tandem Mass Spectrometry. *Chromatographia*, 79(17-18), pp.1069-1083.

[9] Negrusz, A. and Cooper, G. (2013).*Clarke's analytical forensic toxicology*. London: Pharmaceutical Press.

[10] Martin-Reina, J., Duarte, A.J., Cerrillos, J., Bautista, J.D. and Moreno, I. (2017). Insecticide Reproductive Toxicity Profile: Organophosphate, Carbamate and Pyrethroids. *J. Toxins*, 4(1), pp.1-7.

[11] World Health Organisation (WHO) (2010). *WHO recommended classification of pesticides by hazard and guidelines to classification 2009*. Geneva: World Health Organization.

[12] Voelker, R. and Holmes, M. (2015). Pesticide Use on Cannabis. *Cannabis Safety Institute*, pp.1-19.

[13] Dryburgh, L., Bolan, N., Grof, C., Galettis, P., Schneider, J., Lucas, C. and Martin, J. (2018). Cannabis contaminants: sources, distribution, human toxicity and pharmacologic effects. *Br. J. Clin. Pharmacol.*, 84(11), pp.2468-2476.

[14] Ogg, C.L., Hygnstrom, J.R., Alberts, C.A and Bauer, E.C. (2018). Managing Pesticide Poisoning Risk and Understanding the Signs and Symptoms. *The Board of Regents of the University of Nebraska*. Available at: http://extensionpublications.unl.edu/assets/pdf/ec2505.pdf (Accessed 2nd February 2019).

[15] McPartland, J., Clarke, R. and Watson, D. (2000). *Hemp diseases and pests*. Wallingford: CABI Pub.

[16] McPartland, J.M. (1996). Cannabis pests. *J. Ind. Hemp*, 3(2), pp.49-55.

[17] Stone, D. (2014). Cannabis, pesticides and conflicting laws: The dilemma for legalized States and implications for public health. *Regul. Toxicol. Pharmacol.*, 69(3), pp.284-288.

[18] McLaren, J., Swift, W., Dillon, P. and Allsop, S. (2008). Cannabis potency and contamination: a review of the literature.*Addiction*, 103(7), pp.1100-1109.

[19] Lenton, S., Frank, V., Barratt, M., Potter, G. and Decorte, T. (2018). Growing practices and the use of potentially harmful chemical additives among a sample of small-scale cannabis growers in three countries. *Drug Alcohol Depend.*, 192, pp.250-256.

[20] Russo, E. (2016b). Pesticide contamination of cannabis in the legal market. *26th Annual Symposium of the International Cannabinoid Research Society*. Poland, 26 June - 1 July, p.113.

[21] Wurzer, J. (2016). Safety testing of medicinal cannabis. In: *Proceedings of the 2006 Cannmed Conference*. Woburn, MA: Medical Genomics.

[22] Sandler, L., Beckerman, J., Whitford, F. and Gibson, K. (2019). Cannabis as conundrum. *Crop Prot.*, 117, pp.37-44.

[23] EPA (2016). Pesticide use on marijuana. United States Environmental Protection Agency. Available at: https://www.epa.gov/pesticide-registration/pesticide-use-marijuana (Accessed 22nd January 2019).

[24] Feldman, J. (2014). Pesticide Use in Marijuana Production: Safety Issues and Sustainable Options. *Pesticides and You, A quarterly publication of Beyond Pesticides*, 34(4), pp.14-23.

[25] McPartland, J.M. and McKernan, K.J. (2017). Contaminants of Concern in Cannabis: Microbes, Heavy Metals and Pesticides. In: Chandra S., Lata H., ElSohly M. (2017) *Cannabis sativa L. - Botany and Biotechnology*. Cham: Springer.

[26] Russo, E. (2016a). Current Therapeutic Cannabis Controversies and Clinical Trial Design Issues. *Front. Pharmacol.*, 7(309), pp.1-19.

[27] Cuypers, E., Vanhove, W., Gotink, J., Bonneure, A., Van Damme, P. and Tytgat, J. (2017). The use of pesticides in Belgian illicit indoor cannabis plantations. *Forensic Science International*, 277, pp.59-65.

[28] Schneider, S., Bebing, R. and Dauberschmidt, C. (2014). Detection of pesticides in seized illegal cannabis plants. *Analytical Methods*, 6(2), pp.515-520.

[29] Fishel, F.M. (2005). Pesticide Toxicity Profile: Triazole Pesticides. *University of Florida IFAS Extension*, PI-68, pp. 1-4. Available at: https://edis.ifas.ufl.edu/pdffiles/PI/PI10500.pdf (Accessed 27th February 2019).

[30] Sullivan, N., Elzinga, S. and Raber, J. (2013). Determination of Pesticide Residues in Cannabis Smoke. *Journal of Toxicology*, 2013, pp.1-6.

[31] Melamede, R. (2005). Cannabis and tobacco smoke are not equally carcinogenic. *Harm Reduction Journal*, 2(21), pp.1-4.

[32] Raber, J., Elzinga, S. and Kaplan, C. (2015). Understanding dabs: contamination concerns of cannabis concentrates and cannabinoid transfer during the act of dabbing. *The Journal of Toxicological Sciences*, 40(6), pp.797-803.

[33] Mégarbane, B. and Chevillard, L. (2013). The large spectrum of pulmonary complications following illicit drug use: Features and mechanisms. *Chemico-Biological Interactions*, 206(3), pp.444-451.

[34] Subritzky, T., Pettigrew, S. and Lenton, S. (2017). Into the void: Regulating pesticide use in Colorado's commercial cannabis markets. *International Journal of Drug Policy*, 42, pp.86-96.

[35] Moir, D., Rickert, W., Levasseur, G., Larose, Y., Maertens, R., White, P. and Desjardins, S. (2008). A Comparison of Mainstream and Sidestream Marijuana and Tobacco Cigarette Smoke Produced under Two Machine Smoking Conditions. *Chemical Research in Toxicology*, 21(2), pp.494-502.

[36] Lorenz, W., Bahadir, M. and Korte, F. (1987). Thermolysis of pesticide residues during tobacco smoking. *Chemosphere*, 16(2-3), pp.521-522.

[37] Armstrong, B., Carnagey, C., Wu, J., Reddy, S., Ye, J., Qin, F., Wichems, D., Schmidt, C. (2017). Analysis of Pesticide Residues in Cannabis Regulated by Oregon State Using LC/MS/MS. *Perkin Elmer*. Available at: https://www.perkinelmer.com/lab-solutions/resources/docs/APP_Analysis_of_Pesticide_Residues_in_Cannabis_013707_01.pdf (Accessed 16th January 2019).

[38] Staugler, J., Babin, M., Matthews, M., Brittain, M. and Perry, M. (2018). Development of a hydrogen cyanide inhalation exposure system and determination of the inhaled median lethal dose in the swine model. *Inhalation Toxicology*, 30(4-5), pp.195-202.

[39] Gargani, Y., Bishop, P. and Denning, D.W. (2011). Too Many Mouldy Joints – Marijuana and Chronic Pulmonary Aspergillosis. *Mediterranean Journal of Hematology and Infectious Diseases*, 3(1), pp.1-5.

[40] United Nations Office on Drugs and Crime (UNODC) (2009). *Recommended Methods for the Identification and Analysis of Cannabis and Cannabis Products*. New York: United Nations Publications. Available at: https://www.unodc.org/documents/scientific/ST-NAR-40-Ebook_1.pdf (Accessed 5th December 2018).

[41] Alder, L., Greulich, K., Kempe, G. and Vieth, B. (2006). Residue analysis of 500 high priority pesticides: Better by GC–MS or LC–MS/MS?. *Mass Spectrometry Reviews*, 25(6), pp.838-865.

[42] Jansson, C., Pihlström, T., Österdahl, B. and Markides, K. (2004). A new multi-residue method for analysis of pesticide residues in fruit and vegetables using liquid chromatography with tandem mass spectrometric detection. *Journal of Chromatography A*, 1023(1), pp.93-104.

[43] Lacassie, E., Marquet, P., Gaulier, J., Dreyfuss, M. and Lachâtre, G. (2001). Sensitive and specific multiresidue methods for the determination of pesticides of various classes in clinical and forensic toxicology. *Forensic Science International*, 121(1-2), pp.116-125.

[44] Payá, P., Anastassiades, M., Mack, D., Sigalova, I., Tasdelen, B., Oliva, J. and Barba, A. (2007). Analysis of pesticide residues using the Quick Easy Cheap Effective Rugged and Safe (QuEChERS) pesticide multiresidue method in combination with gas and liquid chromatography and tandem mass spectrometric detection. *Analytical and Bioanalytical Chemistry*, 389(6), pp.1697-1714.

[45] European Committee for Standardization (CEN) (2008). Foods of plant origin – Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE – QuEChERS method. Available at: http://www.chromnet.net/Taiwan/QuEChERS_Dispersive_SPE/QuEChERS_%E6%AD%90%E7%9B%9F%E6%96%B9%E6%B3%95_EN156622008_E.pdf (Accessed 28th January 2019).

[46] Daley, P., Lampach, D., Sguerra, S. (2013). Testing Cannabis for Contaminants. *BOTEC Analysis Corporation*. Available at: https://lcb.wa.gov/publications/Marijuana/BOTEC%20reports/1a-Testing-for-Contaminants-Final-Revised.pdf (Accessed 12th December 2018).

[47] Restek (2015). QuEChERS Methodology: AOAC Method. Available at: https://www.restek.com/pdfs/805-01-002.pdf (Accessed 17th May 2019).

[48] Lozano, A., Rajski, Ł., Belmonte-Valles, N., Uclés, A., Uclés, S., Mezcua, M. and Fernández-Alba, A. (2012). Pesticide analysis in teas and chamomile by liquid chromatography and gas chromatography tandem mass spectrometry using a modified QuEChERS method: Validation and pilot survey in real samples. *Journal of Chromatography A*, 1268, pp.109-122.

[49] Ilias, Y., Rudaz, S., Christen, P., Veuthey, J.-L. (2006). Headspace Solid-Phase Microextraction of Pesticide Residues in *Cannabis* Samples. *Chimia International Journal for Chemistry*, 60(12), pp.846-851.

[50] Kusch, P. (2018). Headspace Solid-Phase Microextraction Coupled with Gas Chromatography–Mass Spectrometry for the Characterization of Polymeric Materials. *LCGC North America*, 36(1), pp.52-61.

[51] CEM (2019). Extraction of Pesticides from Cannabis. Available at: http://cem.com/media/contenttype/media/literature/ApNote_EDGE_Extraction_of_Pesticides_from_Cannabis_ap0168v1.pdf (Accessed 17th May 2019).

[52] Craven, C., Wawryk, N., Jiang, P., Liu, Z. and Li, X. (2019). Pesticides and trace elements in cannabis: Analytical and environmental challenges and opportunities. *Journal of Environmental Sciences*.

[53] Steimling, J. and Kahler, T. (2018). Liquid chromatography's complementary role to gas chromatography in cannabis testing. *LCGC North America,* 36(6), pp.36-40.

[54] Ishibashi, M., Ando, T., Sakai, M., Matsubara, A., Uchikata, T., Fukusaki, E. and Bamba, T. (2012). High-throughput simultaneous analysis of pesticides by supercritical fluid chromatography/tandem mass spectrometry. *Journal of Chromatography A*, 1266, pp.143-148.

[55] Lanaro, R., Costa, J., Cazenave, S., Zanolli-Filho, L., Tavares, M. and Chasin, A. (2015). Determination of Herbicides Paraquat, Glyphosate, and Aminomethylphosphonic Acid in Marijuana Samples by Capillary Electrophoresis. *Journal of Forensic Sciences*, 60, pp.S241-S247.

[56] Farrer, D.G. (2015). Technical report: Oregon Health Authority's process to decide which types of contaminants to test for in cannabis. *Oregon Health Authority*. Available at: https://www.oregon.gov/oha/ph/preventionwellness/marijuana/documents/oha-8964-technical-report-marijuana-contaminant-testing.pdf (Accessed 29th June 2019).

[57] Tran, D., Hyland, K.C., Roberts, S., Krepich, S., Winkler, P., Butt, C., Borton, C. (2017). Quantitation of Oregon List of Pesticides and Cannabinoids in Cannabis Matrices by LC-MS/MS. *Food and Environmental*. Available at: https://sciex.com/Documents/tech%20notes/Quantitation-of-Oregon-List-of-Pesticides-and-Cannabinoids.pdf (Accessed 17th May 2019).