Determination of carbamates from food samples by molecularly imprinted polymers

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A dissertation submitted to the Department of Chemistry, University of Venda in fulfilment of the requirements for the degree of Master's in Science in Chemistry

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Co-supervisor: Prof VE Pakade

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Declaration

I, **Nyeleti Bridget Mabaso (student number: 19023428),** declare that this research is my original work and has not been submitted for any degree at any other university or institution. The research does not contain any other person's writing unless specifically acknowledged and referenced accordingly.

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Nyeleti Mabaso Date

List of publications

This dissertation is based on the following papers.

I. **The challenges of template bleeding in molecularly imprinted polymer of pesticides**
Nyeleti B Mabaso, Vusumzi E Pakade, Nikita T Tavengwa
(Manuscript in preparation)

II. **Determination of carbofuran by dummy template molecularly imprinted polymers from grapes**
Nyeleti B Mabaso, Vusumzi E Pakade, Nikita T Tavengwa
(Manuscript in preparation)

III. **Solid phase extraction of pirimicarb from oranges using molecularly imprinted polymers as sorptive material**
Nyeleti B Mabaso, Vusumzi E Pakade, Nikita T Tavengwa
(Manuscript in preparation)

Appendix

IV. **Chiral pesticides: Analysis, fate, and toxicity in the environment**
Herbert Musarurwa, Mpelegeng Victoria Bvumbi, Nyeleti Bridget Mabaso, Nikita Tawanda Tavengwa
(Accepted book chapter)

Contribution of the authors

Paper II. Principal and co-authors were involved in planning and writing of review. Co-authors revised the draft manuscript and made suggestions for improvement.

Paper I. Principal author, involved in planning, synthesis, characterization of the sorbent, adsorption studies and writing of article. Co-authors revised the draft manuscript and made suggestions for improvement.

Paper III. Principal author, involved in planning, synthesis, characterization of the sorbent, sorption studies and writing of article. Co-authors revised the draft manuscript and made suggestions for improvement.

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Abstract

In this work, new clean sorbent that has greater selectivity was used in the determination of pesticides residues in complex matrices at the desired limits of quantification. This work is divided into 3 papers focusing on molecularly imprinted polymers.

Paper I is a review on challenges in elution of the template from molecularly imprinted polymers (MIPs). MIPs are materials that exhibit selective recognition towards the desired template. It possesses advantages of high selectivity, low-cost and low limit of detection. It is a promising alternative for natural antibodies that suffer from poor stability and high cost. However, the MIP has challenges of expensive templates, and the template might not get removed even after extensive washing or the MIP may get damaged during the removal process and this step often requires lengthy optimization which can alter the accuracy of the results. The review summarises the effective ways of washing off the template molecules, paying attention to the effect of washing techniques, pairing agents, and the number of elution cycles applied to achieve satisfactory template removal. It also reviews the use of dummy template and computational chemistry to select appropriate solvent and functional monomer. Finally, challenges and prospects in template bleeding are discussed.

In **Paper II,** carbaryl was used to synthesize dummy template molecularly imprinted polymers (DMIPs) for the determination of carbofuran in grapes. The sorbent was used to selectively extract carbofuran from other closely related compounds. Carbofuran is one of the most toxic carbamate pesticides, and it is banned in most countries. Carbofuran was determined by carbaryl dummy templated molecularly imprinted polymer from grapes. FTIR characterization showed the C=O stretching absorbance in the region of 1714-1730 cm^{-1} between unwashed MIP, NIP, and washed MIP with different intensities. Various parameters affecting the extraction efficiency of the imprinted polymers were evaluated to optimize the selective pre-concentration of carbamates in grape samples. Under optimal condition, the enrichment factors of up to 215 were obtained. This method was successfully applied to quantitively detect carbamates residue in grape sample. The results during application showed that all the grape samples were found to contain no carbamates.

In **Paper III,** pirimicarb is a carbamate pesticide used for controlling pests on vegetables and is used in gardens and household. Pirimicarb was determined using molecularly imprinted polymer from oranges. The MIP material was characterised with FTIR where all the peaks were similar except their intensities. The MIP and NIP were optimised using different parameters and enrichment factor of 19 was obtained. The linear range was 0.25-2.5 mg L^{-1} and percentage recoveries of three carbamates ranges were 69.43 -84.67%. The limit of detection and limit of quantification 0.41-0.61 and 1.24-1.86 mgL^{-1}. The method indicated repeatability as the RSD ranged from 1.37-9.23. The potential of the

developed MIP-SPE method for the selective sample clean-up were investigated in the analysis of oranges. It was found that none of the analytes were detected in the orange samples.

Dedications

This work is dedicated to God for always being there and giving me strength. To my mother Tsakani and father George for always being very supportive and always believing in me. To my daughter Nsuku for giving me strength to push through to the finishing line.

Acknowledgements

- God has been always wonderful to me.
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- I would like to thank my colleagues in the Analytical Chemistry Research Group for making the laboratory an enjoyable place to work in.
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List of abbreviations

ACH	Acetylcholine
ACHE	Acetylcholinesterase
BCF	Bioconcentration factor
DDT	Dichlorodiphenyltrichloroethane
DLLME	Dispersive liquid-liquid micro-extraction
DSPE	Dispersive solid phase extraction
ECD	Electron capture detector
EGDMA	Ethylene glycol dimethacrylate
FID	Flame ionisation detector
FTIR	Fourier -Transform Infrared
GC	Gas chromatography
HPLC	High-performance liquid chromatography
IIPS	Ion imprinted polymer
LLE	Liquid-liquid extraction
LLE-LTP	Liquid-liquid extraction low temperature partitioning
MAL	Maximum allowable limit
MBA	N, N’-methylenebisacrylamide
MD-MSPE	Magnetic dispersive micro-solid phase extraction
MIPs	Molecular imprinted polymer(s)
MMLE	Microporous membrane liquid-liquid extraction
MS	Mass spectrometer
NIP	Non-imprinted polymer
NMJs	Neuromuscular junctions
OCs	Organochlorines

OPS	Organophosphates
PID	Photoionization detector
POPs	Persistent organic pollutants
QuEChERS	Quick, Easy, Cheap, effective, effective, rugged
SDME	Single drop micro-extraction
SEM	Scanning electron microscope
SPE	Solid-phase extraction
SPE	Solid phase extraction
SPME	Solid phase micro-extraction
SULLE	Sugaring-out liquid liquid extraction
TGA	Thermogravimetric analysis
VALLME	Vortex-assisted liquid-liquid micro-extraction

Chapter 1

Introduction and background

This chapter addresses what the study will entail and discusses the existing data. It concludes by giving the outline on how the work is presented in this dissertation.

1.1 Background of the study

Human activities such as farming and industrial processes have contributed to introduction of pollutants such as pesticides into the environment. They enter the environment via accidental spills during transportation, storage (Marican et al., 2017), industrial and commercial facilities (Rajini et al., 2020). Since there is a notable increase in food demand, this has led to newer pesticides introduction with new complex structures in the environment. Therefore, results in bioaccumulation and bio-concentration of the pollutants in which may enhance the persistence of pesticides in the ecosystem. This can cause adverse effects as they are linked with health issues relating to cancer, neurological disorders, obesity (Kumar et al., 2019), Parkinson's disease, reproductive disorder (Sabarwal et al., 2018), birth defects (Sharma et al., 2020), developmental and learning disorders (Mfarrej et al., 2019).

People are poisoned by pesticides when applying them to crops, killing mosquitoes and by eating fruits that have pesticides residues. pesticides are designed to function at a minimal amount that does not affect human health, most data published agrees with the maximum allowable limit set by regulatory bodies. For an example, the maximum allowable limit (MAL) for chlorpyrifos by European union in fruit and vegetables are 0.03-0.05 mg g^{-1} and the detected concentration in apples is 5.4-35.4 µg kg^{-1} (Otieno et al., 2012). China Union has set 0.02 mg kg^{-1} of carbofuran in vegetables and 24.6 and 37.4 ng g^{-1} (Zhao et al., 2019) were detected. Overuse of pesticides will result in high concentration in the environment that are harmful to human, so therefore suitable management is required to minimise the risk.

There are different methods of removing pesticides such as enzymatic biodegradation (Cardozo et al., 2020; Matula et al., 2020), adsorption (Abdelhameed et al., 2018; Suo et al., 2019; Liu et al., 2018) and photocatalytic degradation (Luna-Sanguino et al., 2020; Garcia-Muñoz et al., 2020; AbuKhadra et al., 2020). Adsorption is the best approach among the several approaches as it has higher stability (Ince et al., 2017), low-cost (Saleh et al., 2020) and simple to operate (Awad et al., 2019). The process of adsorption suggests that there should be an adsorbent that binds molecules by physical attraction forces, ion exchange and chemical binding (Demirbas et al., 2008). Preferentially, an adsorbent should be cheap and stable during operation, hence, the introduction of molecularly imprinted polymers (MIPs) that specifically rebind with the target molecules. These methods and adsorbents are reviewed in more details in Chapter 2. Adsorption method was performed in all papers presented in this dissertation. Organophosphates, carbamates, organochlorines, pyrethroids, phenyl urea and phenyl amides have been removed successfully from the environment and their behaviour and toxicities are discussed in detail in **Chapter 2** and Appendix B.

Pesticides are difficult to quantify as they are present in the environment at a very low concentration (Toidŕa et el., 2019), thus extraction methods are required to recover the full amount as most methods that are used for detection are not sensitive or selective. There is therefore a need for pre-treatment before instrumental analysis. Sample preparation is a crucial step as it is the main source of errors in analysis, it determines the precision, accuracy as well as limit of detection (Sajid et al., 2017). The methods of extraction includes dispersive liquid-liquid micro-extraction (DLLME) (Primel et al., 2017; Shamsipur et al., 2016), vortex-assisted liquid-liquid micro-extraction (VALLME) (Jia et al., 2010), solid-phase extraction (SPE) (Liu et al., 2016; Torrento et al., 2019), QuEChERs (Zaidon et al., 2019; Ponce-Robles et al., 2017), liquid-liquid extraction (LLE) (Mesquita et al., 2018; Lakhili et al., 2018; Hassan et al., 2016), dispersive solid phase extraction (DSPE) (Farajzadeh et al., 2017), magnetic dispersive micro-solid phase extraction (MDMSPE) (Rabah et al., 2017), ministurized pipette-tip extraction (Wu et al., 2021), solid phase micro-extraction (SPME) (Lakhili et al., 2018) and single drop micro-extraction (SDME) (Pano-farias et al., 2017). Some of these methods are later reviewed in **Chapter 2**. Miniaturised pipette-tip extraction is the method that was selected in this study as it often replaces classical methods such as liquid-liquid extraction (LLE). Miniaturised pipette-tip provides advantages of low solvent consumption and avoids extended and multistep extraction procedures. The comparison of these methods is reviewed in detail in **Chapter 2.**

1.2 Problem statement

The increase in food demand has led to production of different pesticides with new complex structures in the environment. The overuse or misuse of pesticides especially in agriculture resulted in environmental issues and possible serious health risks to human and animals. Even though pesticides are designed to function at a minimal amount that do not affect human health, the data published is not always in agreement to this fact (Damalas et al., 2011). Therefore, pesticides need to be properly managed as exposure to them is linked to various health disorders such as endocrine disruption, respiratory disorder (Sabarwal et al 2018), headaches, dizziness (Kim et al., 2017), neurological disorder and obesity (Kumar et al., 2019).

Pesticides exist in complex matrices and the sensitivity and selectivity of the most current analytical methods is insufficient for the direct determination of target compounds hence the need for sample pre-treatment before analysis in these complex matrices (Tavengwa et al., 2016). The use of traditional extraction techniques is accompanied by many setbacks. Traditional methods such liquid-

liquid extraction uses a lot of toxic organic solvents which in turn are harmful to the environment. The use of solid-phase extraction does not require phase separation which is associated with errors due to inaccuracy in extraction volumes, and that it uses smaller solvent volume. Recent studies have explored micro-extraction techniques such as miniaturised pipette-tip solid phase extraction to eliminate or reduce solvent consumption. This method exploits microgram of a sorbent, have high enrichment factor (Seidi et al., 2019), it can be carried out faster and more easily than conventional SPE cartridges or with SPE (Kaykhaii et al., 2016). Pipette-tip solid phase extraction has been used in **paper II.**

1.3 Aims and objectives

1.3.1 Aims

-The main aim of the whole project is to determine carbamates by molecularly imprinted polymers from complex matrices.

Paper II aimed at using the sorbent, molecularly imprinted polymers (MIPs), imprinted with carbaryl dummy template to determine carbofuran in grapes with Pipette-tip solid phase extraction

Paper III aimed at using sorbent, molecularly imprinted polymer that is imprinted using pirimicarb to extract this pesticide in complex matrices with solid phase extraction

1.3.2 Specific objectives

Paper II

- To synthesize MIPs using carbaryl as a dummy template.
- Characterization of MIPs using FTIR.
- Optimization of the extraction of carbamates by varying sample pH, adsorbent mass, aspirating/dispensing cycles, types of eluting solvent and elution volume.
- Application of MIPs to grape samples.

Paper III

- To synthesize MIPs using pirimicarb as template.
- Characterization of MIPs using FTIR.
- Optimization of the extraction of carbamates by varying pH, adsorbent mass, sample volume, eluting volume, and concentration.
- Application of MIPs to orange samples.

1.4 Outline of the dissertation

The outline of the dissertation (comprising of five chapters) is presented as follows:

Chapter 1: A general introduction and background to environmental contamination due to presence of pesticides is given. This chapter also spells out problem statement and brings out the motivation of carrying out the research.

Chapter 2: A literature review of carbamates pesticides pollutant.

Chapter 3: Research objectives are provided in this section.

Chapter 4: This chapter lists manuscript (paper I-III) presented for my MSc examination. Results and discussion are presented in each paper.

References: The references arising from introduction and literature review are listed at the end of the dissertation.

Appendix: The appendix has supplementary information of the results of the experiments performed and the book chapter done with colleagues.

Chapter 2

2.1 Literature review

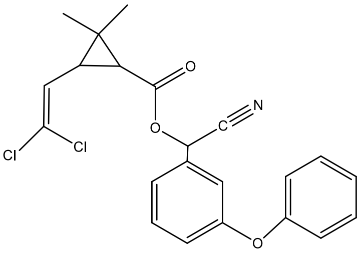
Pesticides were determined from complex matrices using a selective sorbent molecularly imprinted polymer. Different kinds of pesticides were reviewed. Also, different sample preparation techniques and adsorption by different sorbents were critically reviewed.

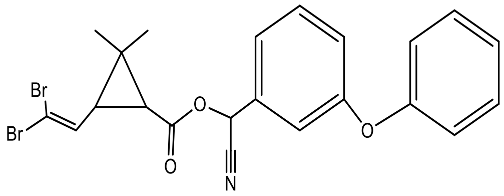
2.1 Pesticides

Pesticides can be classified as herbicides, insecticides, fungicides, nematodes, bactericides, and rodenticides. These groups are applied in commercial agriculture to control and prevent damage of crops and environment from pests, rodents, fungi, weeds, pathogens, microbes, insects, and fungi, thus enhancing the quality of agricultural products and sustaining crop yields by minimizing loss (Pan et al., 2019; Kumar et al., 2019; Liu et al., 2019). They are also used to control or minimise diseases such as malaria (Kim et al., 2017). Therefore, they are important as they secure food security and human health. Pesticides consist of two parts, active and inert ingredients. The active ingredient is used to control the pest, while inert ingredient is solvents that deliver active ingredients (Fan et al., 2015). Pesticides are classified using different criteria. They can be classified based on their formulation where they come in form of powders, gels, gases, granules, and liquids. They are also classified based on the mode of entry; the way pesticides enter the target. The most effective criteria to classify pesticides is based on chemical composition of pesticides and the nature of active ingredients which gives information in physical and chemical properties of pesticides (Yadav, 2015). herbicides and insecticides are two dominant categories of pesticides (Pouokam, 2019). Six groups of pesticides are discussed below namely: organochlorines, organophosphorus, carbamates, pyrethroid, phenyl-urea and phenyl-amide.

2.1.1 Pyrethroids

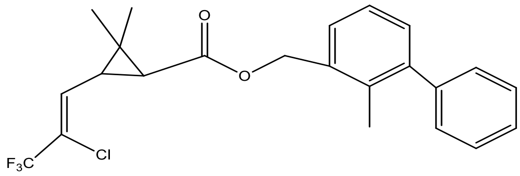
Pyrethroids have been used since 1980. They are obtained from pyrethrin which is derived from *chrysanthemum cinerariifolium* that have strong insecticidal activity by targeting the nervous systems of insects. They are subdivided into groups according to their molecular structures namely: agricultural pyrethroids and urban pyrethroids which are primarily used for agriculture and non-agricultural pest control (Tang et al., 2018). They are also divided into two types according to their structure which are cyclopropane carboxylic acid (Bifenthrin in Figure 2.1) and α-cyano group attached to benzyl carbon (Chedik et al., 2017) (Cypermethrin in Figure 2.1). Pyrethroids are used to remove ticks and flea from dogs (Furlong et al., 2017). They are different from other pesticides as they have more than one chiral centre, extreme hydrophobicity, and broad spectrum high-level insecticidal activity (Bragança et al., 2018). They are non-persistent in the environment and are non-toxic to birds and mammals (Lin et al., 2019). Pyrethroids accumulate in sediments and bioaccumulate in fishes as they are highly lipophilic (Brander et al., 2018). Pyrethroids are lethally toxic to many aquatic organisms and fish (Fan et al., 2017). They have hormone-like activities like that of endocrine disrupting compounds, therefore they are considered a class of endocrine -disrupting chemical (EDCs). These chemicals have different toxicities as they can be observed from Figure 1 that there are different moieties attached to the parent compound and high toxicity of some of the compounds may be due to cyanide group.

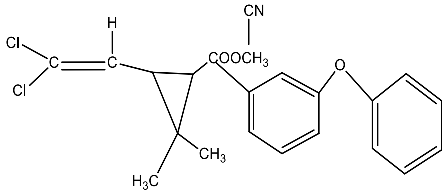




Cypermethrin

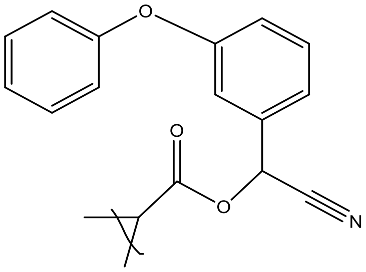
Deltamethrin

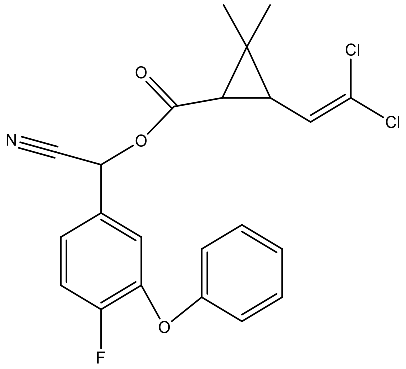




Bifenthrin

λ-cyhalothrin





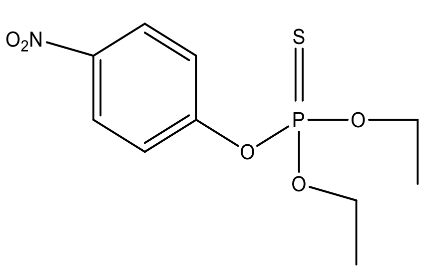
Fenpropathrin

Cyfluthrin

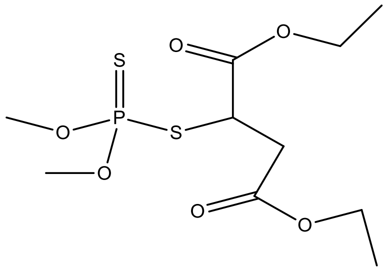
Figure 2.1: Examples of pyrethroid pesticides

2.1.2 Organophosphates

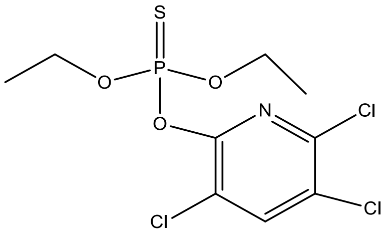
Organophosphates are ester derivatives of phosphorus with a general structure $O=P(OR)_3$, and they have oxygen connected to a phosphorus double bond as shown in structures of Figure 2.2. Organophosphate were brought in the market in 1970 (Murilo et al., 2017). They are applied in agriculture to control pest as well as in residential environment (Wu et al., 2017). They damage the nervous system of mammals and insects by inhibition of acetylcholinesterase enzyme that results in accumulation of acetylcholine, and as a result, stimulate its receptors in synapses (Cui et al., 2018: Phillippat et al., 2018). This is deadly to non-targeted animals and is a threat to human health. It has been reported that they are responsible of 200 000 deaths per year due to their acute poisoning in developing countries (Wu et al., 2017). Organophosphates like diazinon cause diarrhoea, depression, and abdominal cramps (Bagheri et al., 2019). They are widely used due to them rapidly degrading in air and sunlight. They are alternative to organochlorines as they have less persistence (Sidhu et al., 2019).



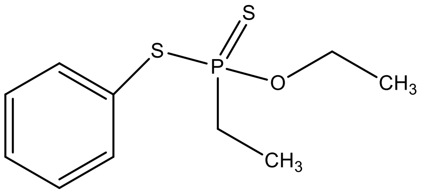
Parathion



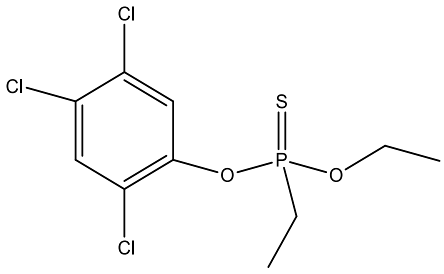
Malathion



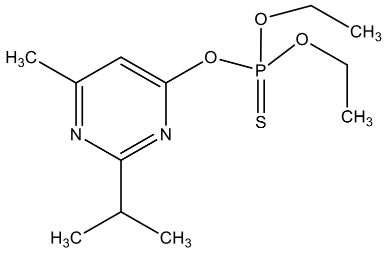
Chlorpyrifos



Fonofos



Trichloronate

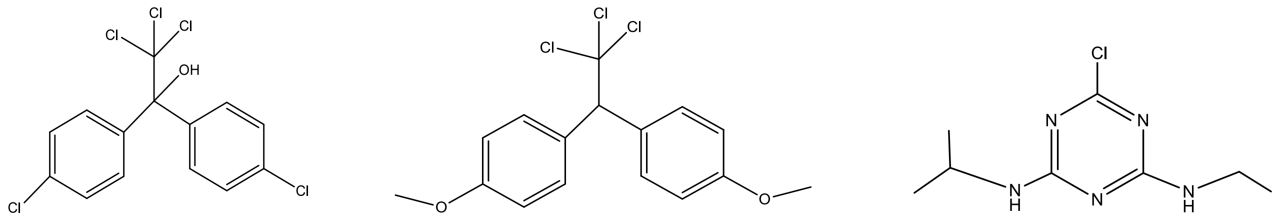


Diazinon

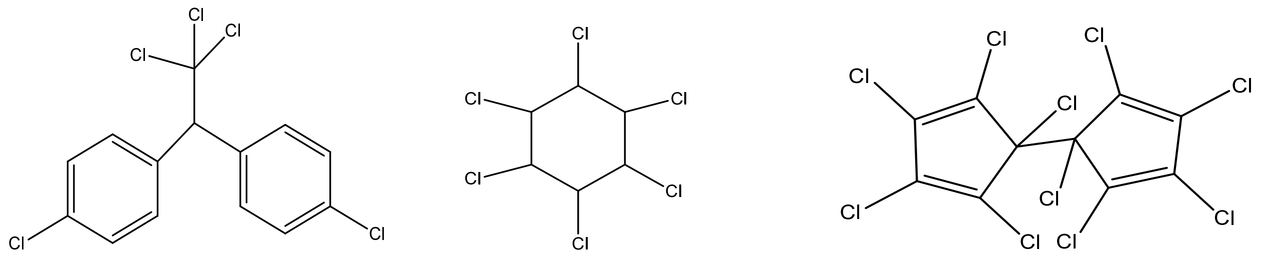
Figure 2.2: Examples of organophosphates

2.1.3 Organochlorines

Organochlorines are synthetic pesticides which at least have one covalently bonded chlorine as shown in Figure 2.3. They are highly effective in eradicating diseases such as malaria and typhus (Docea et al., 2017). They degrade slowly and have high toxicity (Jayaraj et al., 2016). Due to their physico-chemical properties such as high octanol-water partition coefficient, they are distributed and detected in the environment. The high octanol-water partition results in accumulation of organochlorines to the organic matter and leads to contamination of the environment (Hua et al., 2017). Even though they were banned for many years, they still affect wildlife, aquatic biota, and human health (Liu et al., 2017). For example, they are still detected in fish even though they were banned in many countries around 1970 (Wang et al., 2017). Organochlorines are endocrine disruptors which interact with endocrine receptors like oestrogen (Singh, 2016) leading to reproductive effects, cancer, and obesity (Thomas et al., 2017) and neurotoxicity (Wang et al., 2017). They belong to extended persistent organic pollutants (POPs) family and are the most hazardous resulting in damage to the environment (Rani et al., 2017). They have high lipophilicity, low water solubility and low vapour pressure (Man et al., 2018). Their toxicity or behaviour is determined by spatial arrangement, polarity and different parts attached to the parent compound (Ouf et al., 2018).



Dicofol Methoxychlor Atrazine

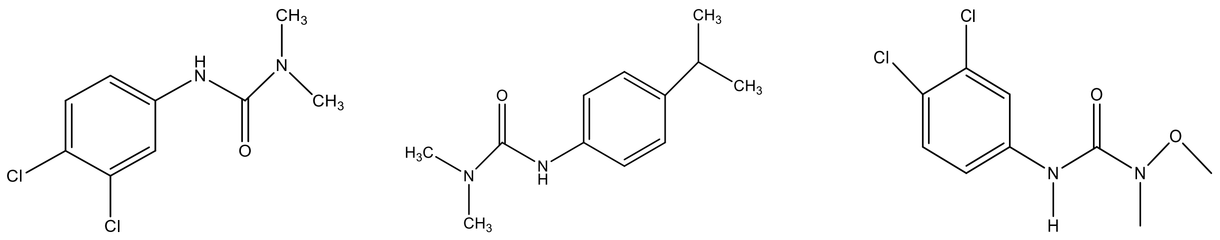


DDT Lindane Dienochlor

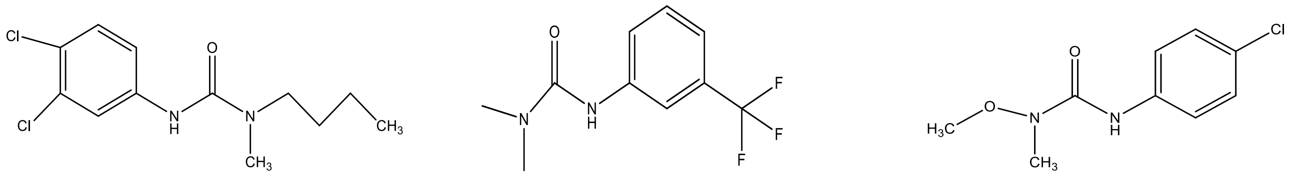
Figure 2.3: Examples of organochlorine pesticide

2.1.4 Phenyl urea

Phenyl urea has a general structure R$_1$–NH–C(O)–NH–SO$_2$–R$_2$. R$_1$ and R$_2$ are generally substituted by heterocyclic rings and their examples are listed in Figure 2.4. They have been commercialised for more than 50 years (Agbaogun et al., 2020) and are applied to control or prevent weed in agricultural crops and railway lines (Wang et al., 2020). When phenyl urea is applied, it may reach distinct destination than their target, resulting in food, water, and soil contamination. Their fate is dependent on their physico-chemical properties and biochemical processes. Phenyl urea are strongly soluble in water and that have caused it to be persistent pesticides, some of phenyl urea pesticides are listed as persistent organic pollutant (Diaw et al., 2017). Understanding the fate of phenyl urea in the environment is important for the accurate assessment of their environmental risk. Diuron phenyl-urea is less persistent and is widely applied in soil. It has been detected in surface and groundwater, and therefore, listed as a priority pollutant by the European Water Framework Directive (Castillo et al., 2016). The widespread of phenyl urea pesticides can cause serious health effects as it is associated with loss of human weight and appetite (Azab et al., 2018) and they are endocrine disruptive and mutagenic towards humans and animals (Agbaogun et al., 2020).



Linuron Diuron Isopruturon

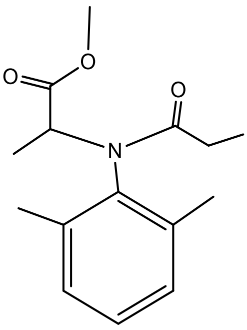


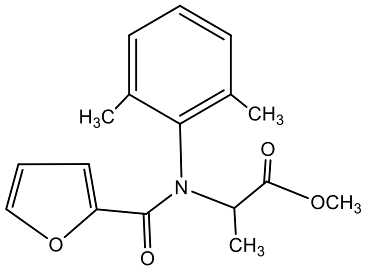
Fluometuron Monolinuron Neburo

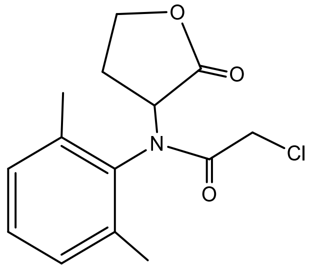
Figure 2.4: Examples of phenyl-urea pesticides

2.1.5 Phenyl amides

Phenyl amides are applied in agriculture to eliminate fungal pathogens (Wei et al., 2016). They inhibit fungal growth by disrupting their RNA synthesis (Kurek et al., 2016). They are also endocrine disruptors and inhibits ubiquitous enzyme succinate dehydrogenase (Wei et al., 2016). Some of the phenyl amides such as benalaxyl, metalaxyl and furalaxyl listed in Figure 2.5 have more than one chiral centre. These compounds contain enantiomers that have different biological and physiological properties (Yang et al., 2020), toxicity, metabolism, and environmental fate (Wang et al., 2017) in a chiral environment. Their enantioselectivity has been observed in preferential degradation of (+)-S-benalaxyl in tomato and cucumber while a faster degradation of R-benalaxyl in soil. It was also observed that metalaxyl-M is the most effective enantiomer, its fungicidal activity is almost 1000 times than that of S-metalaxyl (Yang et al., 2020).


Metalaxyl


Furalaxyl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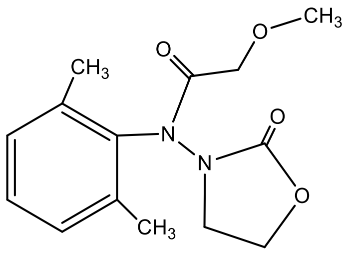
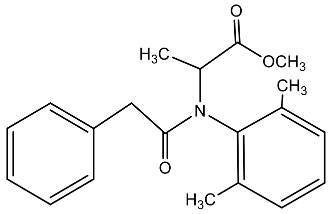

Oxadixyl


Benalaxyl

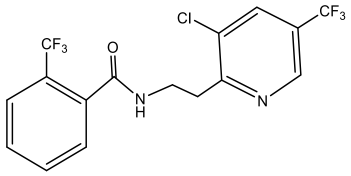
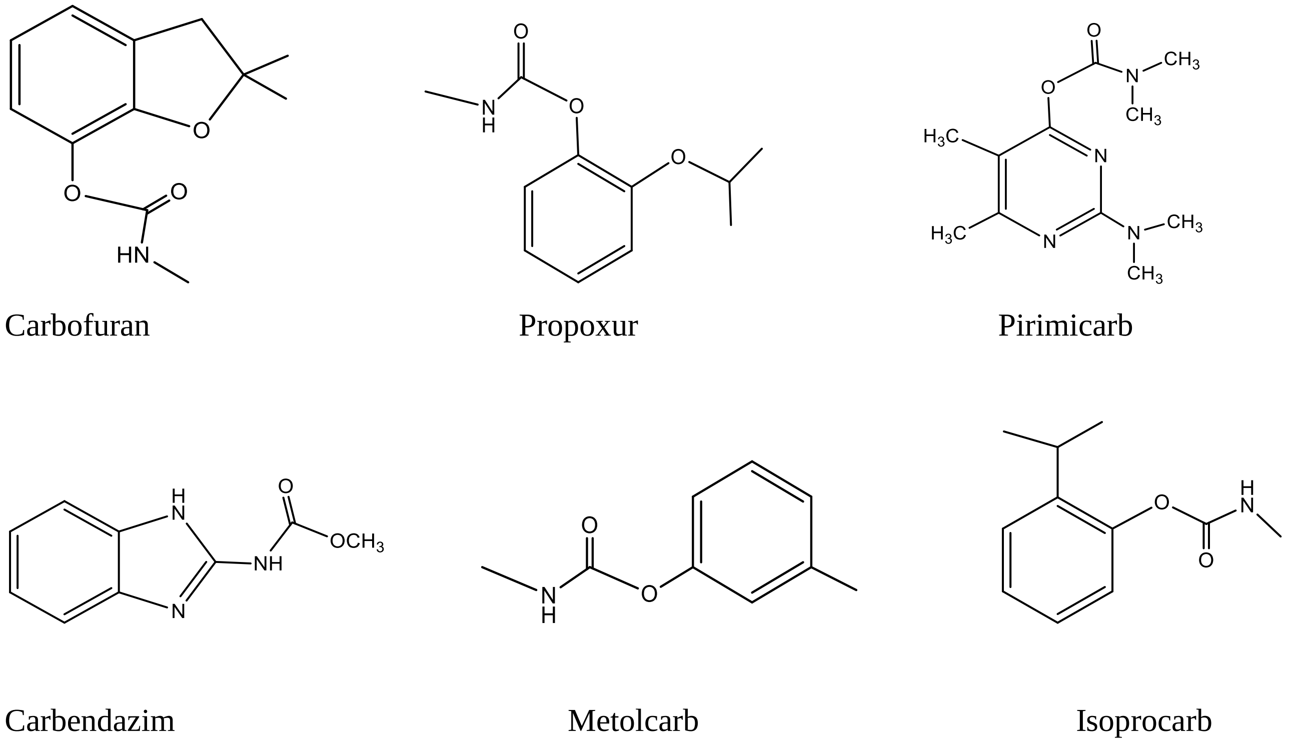

Fluopyram

Figure 2.5: Examples of phenyl amides pesticides

2.1.6 Carbamates

Carbamates are derivatives of carbamic acids, they are made of thioesters (R_1-S-CO-NR_2R_3) and esters (R_1-O-CO-NR_2R_3) as indicated by the structures in Figure 2.6. They have the same toxic mechanism as organophosphorus, but less neurotoxic effect. Carbamates have short environment persistent and mammalian toxicities (Wei et al., 2018). Due to excessive use, they remain in crops, vegetable, and fruits. Environmental water is contaminated by carbamates through soil seepage, spraying and storage of water. They get their toxicity from inhibition of the acetylcholinesterase enzyme (Gupta et al., 2008) and lead to imbalance of free radicals and antioxidants in the body which leads to cell and tissue damage. It has been suspected to be carcinogenic (Chullasat et al., 2020). The toxicity of carbamates differs from one carbamate to another. For example, some carbamates are used as poisonous gases, and some are used as drugs in humans and domestic animals. Therefore, there are carbamates with minimal toxicity and ones that are very toxic. It is not correct to extrapolate the toxicological data from one carbamate to others even if they come from the same family. There is a need to study the toxicity of carbamate individually (Iturbe-Requena et al., 2019) and developing an accurate and reliable multi-residue method for monitoring carbamates in complex matrices. This is important to ensure the safety of the environment and humans.



Carbofuran Propoxur Pirimicarb

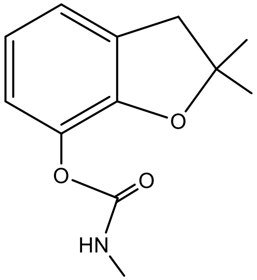
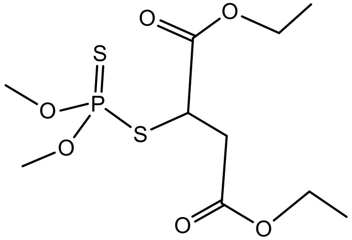
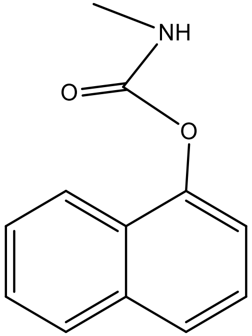
Carbendazim Metolcarb Isoprocarb

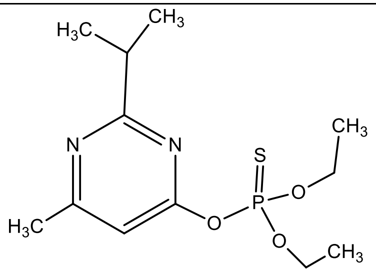
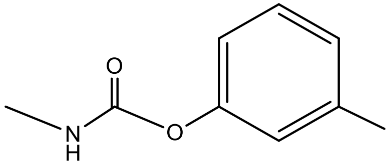
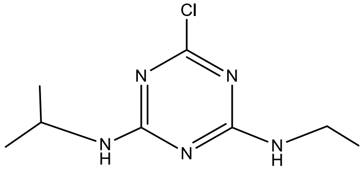
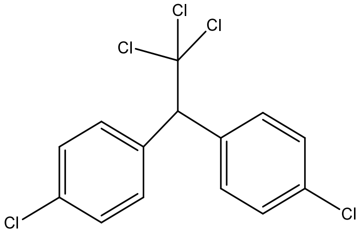
Figure 2.6: Examples of carbamates

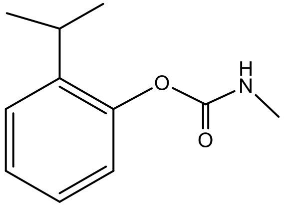
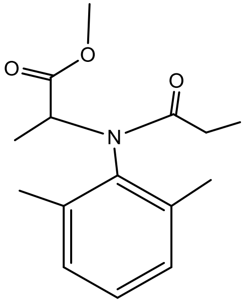
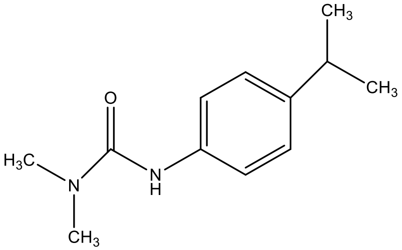
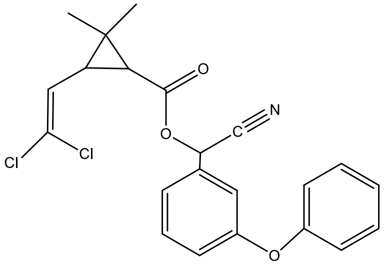
2.2 Fate of pesticides

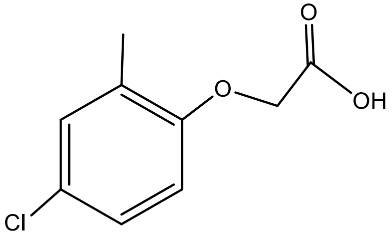
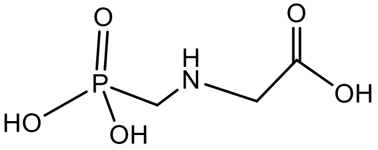
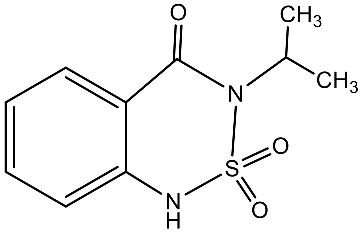
Table 2.1 represents physico-chemical properties of some of the pesticides from different groups. The behaviour of pesticides such as degradation is related to their physico-chemical properties in the environment. Adsorption capacity, texture and permeability of the soil play a role in behaviour of the pesticides in the environment (Menchem et al., 2017). Pesticides can become volatilised into the atmosphere, degraded, or affect the non-target organisms depending on their physical and chemical properties (Muhammed 2017). N-octanol / water partition coefficient (Kow) is used to estimate the transport and the exposure of pesticides through different compartments, bioconcentration factor and acute toxicity in aquatic organisms (Saranjampour et al, 2019). Metalaxyl, in Table 2.1 has a high solubility, therefore can move easily to reach surface and underground water (Islam et al., 2020) and carbofuran which is the most toxic carbamate has high solubility, and this is a concern as it can move easily (Abdelhaleem et al., 2020) and contaminate ground water as well. Cypermethrin have high n-octanol coefficient as indicated in Table 1, this compound does not remain in water but will partition into the sediments (Eljarrat et al., 2020).

Table 2.1: Physico-chemical properties of pesticides

Pesticide	Pesticide category	Structure	Molecular weight (g mol^{-1})	Water solubility	k_ow	pka
Carbofuran	Carbamates		221.25	700	2.32	3.78 2.16
Malathion	Organophosphates		330.40	143	2.36	-
Carbaryl	Carbamates		201.22	40	2.36	10.4

Diazinon	Organophosphates		304.35	60	3.81	2.6
Metolcarb	Carbamates		165.19	2.010	1.70	14.77
Atrazine	Organochlorines		215.68	33.0	2.61	101
DDT	Organochlorines		354.50	5.5×10^{-3}	6.91	-

Isoprocarb	Carbamates		193.24	270	2.31	-
Metalaxyl	Phenylamides		279.33	8 400	1.65	-
Diuron	Phenyl-urea		233.09	42.0	2.68	-
Cypermethrin	Pyrethroids		416.3	4×10^{-3}	6.60	-

2-methyl-4-chlorophenoxyacetic acid (MCPA)	Phenoxy		200.62	29.39	-0.81	3.9
Glyphosate	Organophosphorus		169.07	10.50	-3.2	<2 2.6 5.6 10.6
Bentazone	Thiadiazine		240.28	570	-0.46	3.3

-Means pka value not available

2.3 Toxicology of pesticides

Even though pesticides ensure food security, there are health concerns as they cause environmental and food contamination. This is a problem in world's public health as it damages ecosystem function, non-target species and food webs (Liu et al., 2019, Bhandari et al., 2019). Excessive consumption and misuse of synthetic pesticides results in fruits and vegetables that does no longer have the same natural taste, size, colour, and smell and some can be found in abnormal sizes (Mfarrej et al., 2019). People also develop headache, loss of consciousness, diarrhoea because of exposure to pesticides depending on the type used (Mubushar et al., 2019). Exposure to pesticides can also result in health issues related to cancer, neurological disorders, obesity, allergic wheezing, and endocrine problems (Kumar et al., 2019). Pesticide residues have been found in cereals, fruits, vegetables, tea, and seafood. It has been reported that 25 million people who work in agricultural fields have been accidental poisoned by pesticides (Mfarrej et al., 2019) each year, and 300 000 died from pesticides poisoning yearly throughout the world (Liu et al., 2019).

Therefore, suitable management and effective control of pesticides is very important. International environmental regulatory bodies such as world Health Organisation (WHO) and European Union (EU) have set allowable acceptable (MAL) limit for pesticide residue and are summarised in Table 2.2.

Table 2.2: Maximum allowable concentration of different pesticides in different compartment

Regulatory Organisation	Pesticide(s)	Maximum allowable limit	Matrice(s)	Reference
WHO	Carbofuran	3 µg L^{-1}	Drinking water	Mayakaduwa et al., 2016
	Atrazine	0.22 µg L^{-1}		Ehrampoush et al., 2017
	Diazinon	0.1 µg L^{-1}		
EPA	Atrazine	40 µg L^{-1}	Drinking water	Mayakaduwa et al., 2016
	Diazinon	9×10^{-6} mg L^{-1}		Ehrampoush et al., 2017
DOH	Diphenylamine	10 mg kg^{-1}	Pears and apple	South African Department of Health DOH
	Myclobutanil	0.05-0.5	Cucurbits, dry beans, and pears	
Canadian water quality guidelines	Carbaryl	0.2 µg L	Fresh water	Yang et al., 2013
	Carbofuran	90 µg L^{-1}	-	Schöntag et al., 2019
European Union directive	Individual Pesticides	0.1 µg L^{-1} 0.5 µg L^{-1}	Drinking water	Chullasat et al., 2020

	Total pesticides Metalaxyl	1.5 mg kg $^{-1}$	Swiss chad	Kabir et al., 2018
Codex Alimentarius	Disulfoton	0.01 mg kg^{-1}	some food samples, including milk	do lago et al., 2020
	Pirimiphos	0.01 mg kg^{-1}		
	Chlorpyrifos	0.02 mg kg^{-1}		
China	Metalaxyl	50 μg kg^{-1}	Potato samples	Yu et al., 2018
	Azoxystrobin	100 μg kg $^{-1}$		
MFDS	Metalaxyl	20.0 mg kg^{-1}	Swiss chard	Kabir et al., 2018

WHO = World Health Organisation, EPA = Environmental Protection Agency, DOH = Department of Health and MFDS = Korean Ministry of Food and Drug Safety

2.4 Concentration of pesticides in different matrices

Table 2.3 represents concentration of different pesticides in different matrices in different countries. Some of the pesticides were banned years ago, but their high persistence made them to be still detected in different environmental compartments. For example, parathion and organochlorines (DDTs) were banned since 1972 but are still detected in the environment (Kafaei et al., 2020; Tao et al., 2021). Continuous farming of crops increases concentration of pesticides even if low concentration of pesticides were used. Therefore, pesticides need close monitoring.

Table 2.3: Concentration of pesticides in different matrices

Pesticide	Concentration	Matrice(s)	Reference
DDE	253.58 ± 4.78 μg kg^{-1}	Beef	Nuapia at al., 2016
Dieldrin	0.99 ± 0.33 μg kg-^{1}	Water	Ogbelde et al., 2019
Indane	18.51 ± 0.16 mgL-^{1}	Female Blood	Bayayeva et al., 2016
Endrin	37.57 ± 0.9 mgL-^{1}	Female Blood	Bayayeva. et al., 2016
DDT	1.328 μg kg^{-1}	Fishe (*Clarius Werneri)*	Umulisa et al., 2020
DDT	68 μg kg^{-1}	Lake Edward	Umulisa et al., 2020
DDT	49.74 μg kg^{-1}	Soil	Sun et al., 2016
HCM	236.4 ng L^{-1}	Water (stream, rivers and tap water)	Elibariki et al., 2017

Heptachlor	1.1–15.8 ng L^{-1}	Water (stream, rivers and tap water)	Elibariki et al., 2017
Carbaryl	1737 µg L^{-1}	Water	Schroeder-Spain et al., 2018
Metalaxyl	0.49 mg L−1	Ground water	Islam et al., 2020
Diuron	0.02 to 408 µg L^{-1}	Surface and groundwater	Sposito et al., 2018
lambda-cyhalothrin	13.4 to 18.1 µg kg^{-1}	Fish	Clasen et al., 2018
Tebuconazole	23.8 to 39.9 µg kg^{-1}	Fish	Clasen et al., 2018

2.4 Source and discharge of pesticides

Pesticides are sprayed using different equipment that can dispensed further away from the target. This leads to aquatic life being affected because of deposition of pesticides by rain and wastewater (Mfarrej et al., 2019). It is estimated that 99% of the pesticides sprayed goes to the environment while only 1% pesticides reach the target. This leads to soil contamination, water pollution and resistance by pathogens (Hayat et al., 2018). Pesticides can enter to the water bodies through different mechanisms which are leaching, erosion and runoff. Six processes that can move pesticides are leaching, diffusion, volatilization, erosion, and runoff.

2.5 Sample preparation techniques

Sample pre-treatment is important as pesticides occur in complex matrices, and the target analyte needs to be separated to reduce interferences during analyses (Li et al., 2015). Most methods do not allow direct determination from complex matrices, and that there is a limited number of analytical techniques that are sensitive enough for directly application in trace pollutants (Rykowska et al., 2018). The challenge is some of the existing methods have a lot of preparatory steps which introduce errors on the results. The aim is to design methods that uses fewer toxic chemicals and have better accuracy.

Classical methods such as liquid extraction became less desirable to researchers as it involve using lots of organic solvents which are harmful to the environment and human beings (Li et al., 2017). It is replaced by solid phase extraction. Solid phase extraction has been used to pre-concentrate pesticides in different matrices for example thirteen pesticides has been determined in ground and river water (Kharbouche et al., 2019). It also has been used to extract organophosphorus from fruit juice and water samples (Amiri et al., 2020). This method uses small organic solvents and gives high enrichment factor. Modified SPE such as solid phase micro-extraction with more advantages compared to classical SPE has been developed and used to pre-concentrate pesticides.

2.6 Liquid-liquid extraction of pesticides

Liquid-liquid extraction is a classical method used to extract analytes of interest from the feed (matrices). It is widely used due to simplicity and compatibility with most analytical instruments. It was first applied by Hemberg and Westlin (1973) to purify cytokinins. It also has been used in pesticides sample preparation (Table 2.4). T1his method is based on the partioning of analytes in a certain ratio between two immiscible solvents (Li et al., 2017). Therefore, itmenables separation of two components because of their differences in solubilities (Lee et al., 2009). The immiscible liquids are usually water (polar) and organic solvents (non-polar) (e.g., ether, chloroform, n-hexane, acetonitrile, and acetone). Polar compounds will be more attracted to polar solvents and non-polar will be attracted to non-polar solvents, “like dissolve like rule”. The solvent requirement in LLE is that it should be able to dissolve the analyte. The analyte should possess high partion coefficient (Nair et al., 2017) and larger distribution ratio to have a high recovery rate and enrichment factor (Rao et al., 2019). LLE is a less desirable method as it is time-consuming, tedious, provides low enrichment of analyte (Farajzadeh et al., 2015), uses large amounts of hazardous organic solvents that are potential threat to human health (Farajzadeh et al., 2016), and hazardous to the environment. The new trends in sample preparation promote green technology that prohibit the use of hazardous chemicals and the simplification and miniaturization to protect the environment and they overcome some of the LLE shortages. The improved methods include solid-phase micro-extraction, dispersive liquid-liquid micro-extraction (DLLME), air-assisted liquid-liquid micro-extraction, solvent bar liquid-phase micro-extraction, single drop liquid phase extraction (SD-LPME), hollow fibre liquid phase micro-extraction air-assisted liquid-liquid microextraction and counter current salting-out homogeneous liquid-liquid extraction. Table 2.4 indicates the comparison of LLE with advanced LLE methods in terms of volume used, limit of detection and selectivity. It can be observed in the table that the traditional liquid extraction uses high solvent volumes.

2.7 Comparison of liquid-liquid extraction techniques

Comparison of different liquid-liquid extraction that were used to pre-concentrate pesticides in different matrices is shown on Table 2.4. It can be observed that classical LLE uses a lot of solvent and analysis is commonly carried out using gas chromatography coupled with MS detector.

Table 2.4: Extraction of pesticides by LLE based methods

Pesticide name	Pre-treatment method	Analytical Technique	Sample matrix	Extraction solvent and volume	LOD	Reference
Aldicarb Carbofuran Carbaryl	LLE-LTP	HPLC-UV	Water	Acetonitrile (40 mL)	5.0 and 10.0 µg L^{-1}	Goulart et al., 2010
Malathion Imidacloprid Triadimefon Diazinon	SULLE	HPLC-MS/MS	Fruit and berry juices	Acetonitrile (0.6 mL)	3.10-3 mg L^{-1} 3.10-2 mg L^{-1} 3.10-4 mg L^{-1}	Timfeeva et al., 2017
12 pesticides	LLE-DSPE	GC-MS	Blended oil	MECN (10 mL)	0.0006-0.0012 mg kg^{-1}	Fang et al., 2017
5 pyrethroid pesticides	LLE-DLLME	GC-FID	Fruit juices and fresh grape	N-hexane for LLE 20 µL of 1,1,2-trichloethane	0.1-15 µg L^{-1}	Farajzadeh et al., 2014
Diazon Malathion	Dispersive-LLE	GC-FID	Water	Methanol containing butyl acetate	0.1 µgL^{-1}	Hassan et al., 2016
27 pesticides from 6 groups of pesticides	DLLME	GC-MS	Wine	Chloroform (60 µL)	0.025-0.88 µg L^{-1}	Chen et al., 2016
9 fungicides	Me-VADLLME	GC-ECD	Chrysanthemum	Acetone (1.5 mL)	(0.005-0.05) ×10^{-3} mg kg^{-1}	Xue et al., 2015

				Toluene (50 µL)		
Pesticides from different classes (24 different pesticides)	DLLME	GC-MS	Pumpkin seeds	Chloroform (50 µL)	0.01-12.17 µg kg^{-1}	Andraščíková et al., 2016
Multi-class pesticides	LLE	GC-MS/MS	Edible oils	Mixture of Ethyl acetate and acetonitrile (5ml)	-	Zhao et al., 2019
Pesticides	MLLE	GC-FID	Red wines	-	0.03–0.4 µg L^{-1}	Hyötyläinen et al., 2004

-LOD not specified

2.8 Dispersive liquid-liquid micro-extraction

Dispersive liquid-liquid micro-extraction (DLLME) involves extraction of analyte from the feed. This is achieved by injecting a mixture of dispersive solvent and extracting solvent into aqueous phase sample, and result in emulsion. The aqueous phase should be immiscible to both extracting and dispersive solvent. Then micro-drops form due to extractant solvent which is subsequently centrifuged and the fine drops of the extractant phase are sedimented at the bottom of the tube (Campilo et al., 2016) as shown in Figure 2.7. This technique was introduced by Rezaee (2006) for the preconcentration of organic analytes from aqueous matrices (Rykowska et al., 2018). DLLME has been used successfully in determination of pesticides as indicated in Table 2.5 and had achieved good enrichment factors. DLLME is better than other methods in terms of enrichment factors. It was also observed by (Xiong et al., 2008) that (EFs) of DLLME (176–946) was higher than that of HF-LPME 27–530) for determination of organosulfur pesticides.

DLLME has limitations that results due to dispenser solvent requirement (Sereshti et al., 2019). To overcome this drawback, extensive modification methods such as vortex-assisted and air assisted to get dispersion without centrifugation or to perform the extraction without a dispenser solvent has been used (Li et al., 2019; Soltani et al., 2021; Zhao et al., 2021; Tışlı et al., 2020) to extract pesticides from complex matrices. These methods have brought advantages of simplicity on the operating, and high enrichment factors (Farajzadeh et al., 2016).

Table 2.5: Enrichment factors of DDLME in determination of pesticides

Pesticide	Enrichment factor	Reference
Malathion	200	Ramin et al., 2019
Pyrethroid	176-213	Fan et al., 2017
Flumethrin, bifenthrin and silafluofen	108-125	Li et al., 2020
Acidic pesticides	1232-1532	Mogaddam et al., 2020
12 pesticides	264-464	Farajzadeh et al., 2017
5 pyrethroids	265–360	Deng et al., 2019
Pesticides	320 and 445	Jouyban et al., 2020
Pyrethroids	265–360	Deng et al., 2019

Organochlorine	189 to 269	Xu et al., 2017
5 carbamate pesticides	147–217	Anvar et al., 2020

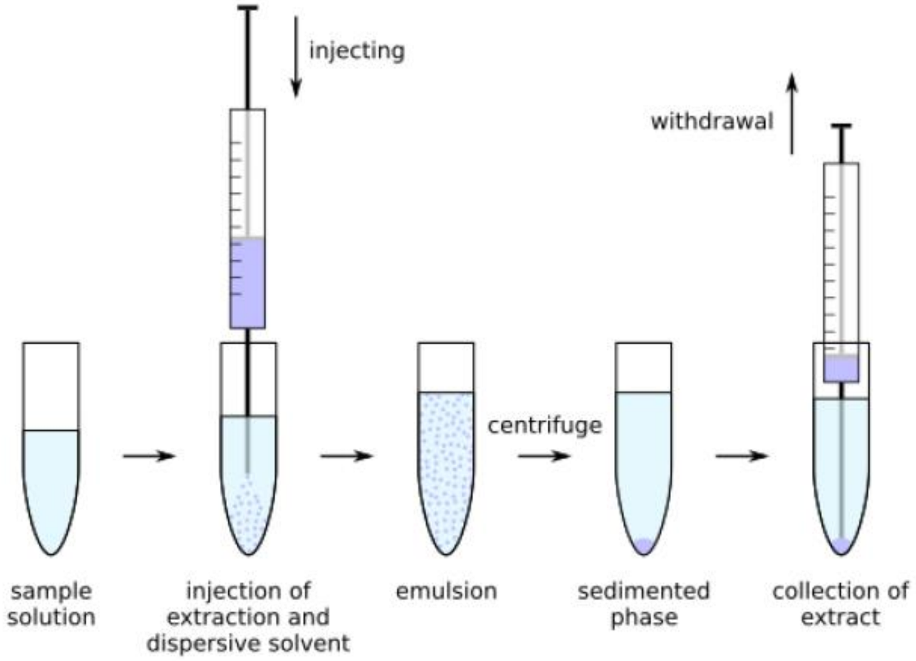


Figure 2.7: Dispersive liquid-liquid micro-extraction method

2.9 Single drop liquid phase extraction

Single drop liquid micro-phase extraction (SD-LPME) was first introduced by Liu and Dasgupta (1996). It is used to pre-concentrate analytes using micro-litre volume of a single organic droplet (An et al., 2017) which serves as an extraction phase. The extraction phase (single organic droplet) is located at the tip of a micro-syringe needle (Hansen et al., 2020). This is exposed directly in the liquid matrix, and the resulted micro-drop from extraction is withdrawn and transferred to the instrument (Mafra et al., 2019).

This method offers advantages of low organic solvent consumption and automation potential (Yamini et al., 2019). This method has been coupled various instruments, mostly GC, for determination of pesticides in aqueous samples (Pano-Farias et al., 2017), triazole fungicides from fruit juice and vegetable samples (Abolghasemi et al., 2020) and for determination of organophosphorus pesticides in soil (Salemi et al., 2013). However, it has limitation of instability of the micro-drop in the direct-immersion mode under high stirring rates, elevated temperatures, and long micro-extraction times (Mafra et al., 2019).

2.10 Solid phase extraction

Although SPE has overcome some shortages of LLE, cartridge obstruction is a problem. Solid phase extraction is a widely used sampling technique that is used for pre-concentration and clean-up of trace pollutants in the environment (Andreda-Eiroa et al., 2016). Pesticides are present in low concentrations, and they also exist in complex matrices, hence sample preparation before analysis is an essential step. Solid phase extraction uses the sorbent to isolate the analyte from a given sample (Chisvert et al., 2019). It involves introducing a liquid sample to an extraction cartridge in a small syringe-shaped container (Pichon 2016) as shown in Figure 2.8. The cartridge contains adsorbent and analyte from the complex matrices. The sorbents that were used for pesticides extraction were Silica-bonded sorbent C18 (Srivastava et al., 2017), polymeric resins (Zhang et al., 2020) and metal-organic frameworks (MOFs) (Ma et al., 2018).

The sorbents such as molecularly imprinted polymers which are improved were selective materials introduced. They are easy to prepare, and they can selectively recognise target molecule in preference to other closely related compounds (Marc et al., 2018). SPE is replacing liquid-liquid extraction as it does not require phase separation which is associated with errors due to inaccuracy in extract volumes. and it is more selective and uses smaller solvent volume (Vasconcelos et al., 2017).

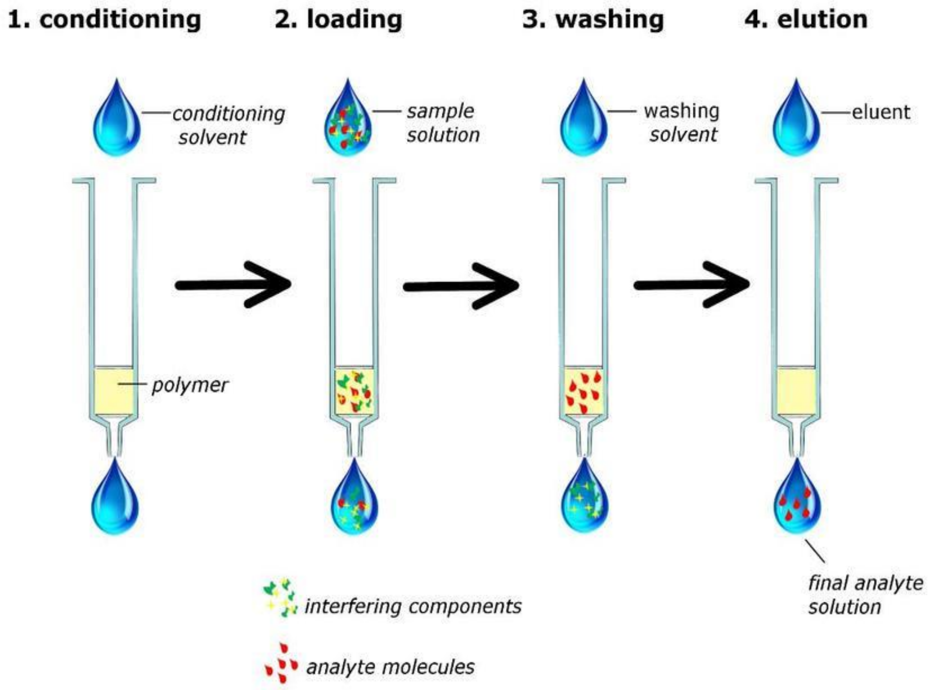


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Figure 2.8: Basic procedure of solid phase extraction

2.11 Miniaturised pipette-tip solid extraction

Pipette-tip solid-phase extraction (PT-SPE) is a miniaturized sample pre-treatment technique that uses a small sorbent and organic solvent to purify and concentrate samples like biological samples (Chen et al., 2019; Zhang et al., 2018; Mirzaee et al., 2021), purification of diverse analytes such as proteins and peptides in various matrices (Amini et al., 2020). It was also used to pre-concentrate pesticides in Chinese yams (Wu et al., 2021), pre-concentration of malathion and parathion from environmental samples (Esrafili et al., 2020) and for phenoxy herbicides in complex samples (Su et al., 2020). The sorbent is loaded in a pipette-tip and the experiments are carried out by dispensing/aspirating the sample like in **Paper II**. It is the method of choice due to advantages such as shorter operation time (Zhang et al., 2018), lower cost (Yan et al., 2019), lower sample volume and combination of extraction and purification step (Bagheri et al., 2019), simplicity of handling and lower detection limits (de Oliveira et al., 2016). The efficiency of PT-SPE is dependent on the sorbent type (Yan et al., 2019). The combination of MIP and PTSPE offers better recoveries, selectivity, and detection limits (Wu et al., 2020). This gave motivation to prepare **paper II**. Figure 2.9 represents the pipette-tip solid phase micro-extraction process.

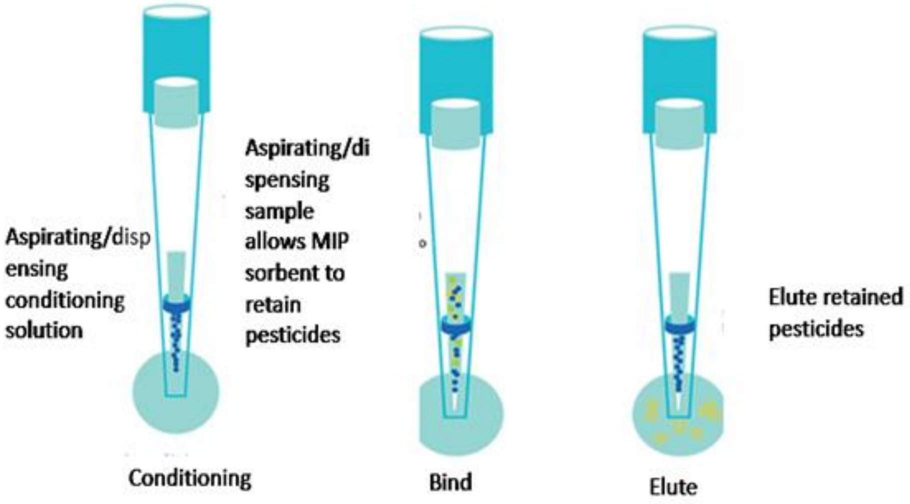

Figure 2.9: Pipette-tip solid phase micro-extraction

2.12 Extractive sorbents

There is a list of sorbents in literature has been used to extract pesticides in different matrices namely activated carbon, carbon nanotubes and electron spun fibres. Activated carbon (AC) is synthesized by pyrolyzing the material at temperatures 600-900 °C or oxidising it at temperatures above 250 °C. The material can also be chemical activated using acid, strong base or salt and the carbon is subjected to lower temperatures (250–600 °C). Activated carbon adsorbent has found to be most effective in removal of organic pesticides (Derylo-Marczewska et al., 2017) and it has also showed greater potential for the treatment of wastewaters coming from agricultural waste (Mohammad et al., 2017). The percentage removal or removal efficiency of pesticide by activated carbon is good for example, removal of oxamyl by silkworm faeces is 99.48 % and mesoporous activated carbon from starch 11 pesticides was still over 80% which are higher than commercial activated carbon (AC), graphitised carbon black (GCB), C18, and primary secondary amine adsorbent (PSA) (Suo et al., 2019). They have advantages of low cost. (Mohammad et al., 2017). Even though activated carbon has been reported to remove numerous pesticides in the environment high cost activated carbon (Alalm et al., 2018), lack of selectivity, low adsorption capacity and limitation of surface functional groups limits its application (Kuang et al., 2020).

Carbon nanotubes are made from graphene sheets of nanometre diameter that are wrapped forming a cylindrical shape. They are synthesized using Arc discharge, laser ablation, chemical vapour deposition, flame synthesis, and saline solution method. They can be classified as single (SWNTs) and multi-walled (MWCNTs) carbon nanotubes. SWNTs has a diameter from 0.4 to > 3nm while MWCNTs has a diameter ranging from 1.4 to 100nm (Rahman et al., 2019). Both nanotubes have been used for pesticides determination (Zhu et al., 2019) and (Xu et al., 2018). Their properties like high chemical stability (Sipa et al., 2018), large specific surface area (Zhao et al., 2020), interactions with different chemical groups, (Alshehri et al., 2016; do Lago et al., 2020), tubular structures (Zhao et al., 2018), thermal conductivity (Moreno et al., 2018) enable them to be used as adsorbents. During adsorption they interact with the analyte via hydrogen bond, hydrophobic, dipole dipole and covalent bonding (Barbosa et al., 2017). Carbon nanotubes have been used to remove pesticides from complex matrices. It has showed excellent adsorption capability as percentage of up to 100 % was achieved (Dehghani et al., 2017). High backpressure that restricts sample flow limits the application of carbon nano tubes (Zhao et al., 2018).

2.13 Molecular imprinted polymer

Molecularly imprinted polymers (MIPs) are materials that have original design in terms of shape and functional groups of that of a template or a target molecule. MIPs are synthesised through a technique that involves interaction of a functional monomers in an appropriate solvent with a template via a covalent, semi-covalent, and non-covalent connections to form a polymer network around the template (complexation). The complex is then polymerised in the presence of cross-linker (Figueiredo et al., 2016), and thereafter, a template is removed by washing with a polar solvent, and as result leaves material that has a binding site that can selectively and specifically bind with the similar molecule or the target molecule from the closely related analytes (Dinc et al., 2019; Ansari and Mosoum, 2019; Cantarella et al., 2019). Figure 2.10 shows imprinting process. It shows copolymerisation of a functional monomer and a cross-linker and initiator. For imprinting technique to be successful, pre-polymerisation reagents should be successfully selected. Pre-polymerisation reagents include porogenic solvents, functional monomers, cross-linkers, and radical initiators.

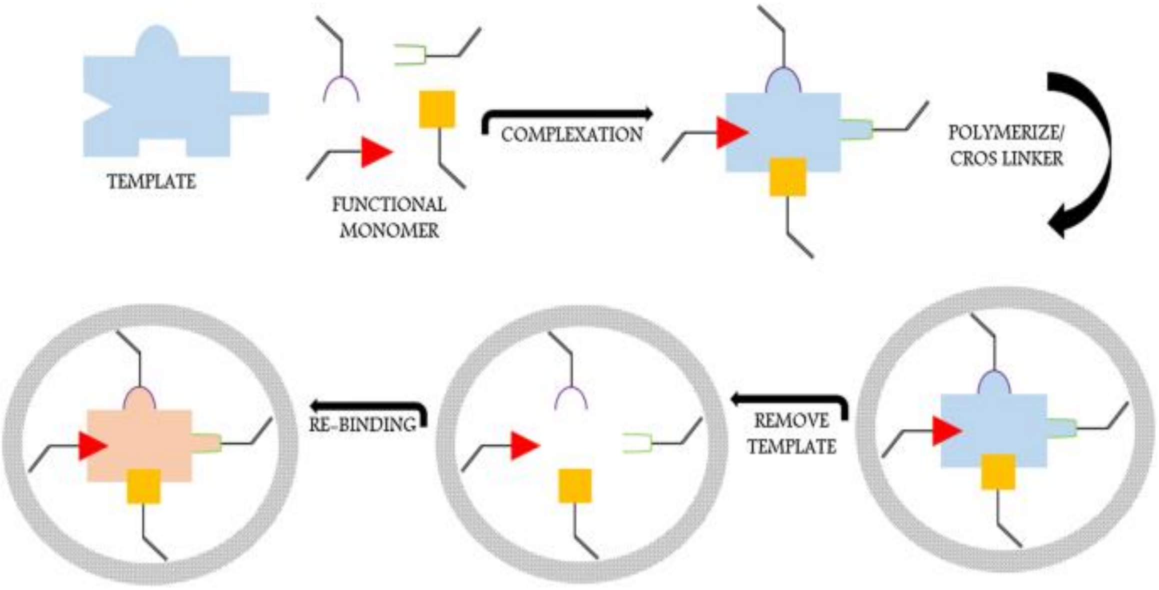

Figure 2.10: Schematic representation of consecutive steps of MIP formation

Molecular imprinting has gained popularity to researchers as they offer several advantageous properties such as high chemical stability, selectivity (Udomsap et al., 2018), recognition specificity, structure predictability (Sajini, et al, 2019), physical robustness (Zhang et.al., 2019), easy availability (Yang et al., 2019), a chance of not doing extensive pre-treatment when analysing complex matrices (Guoning et al., 2019) and that they can be developed for any template. Due to these properties, MIPs have found many applications from various fields. Protein-imprinted polymers have been successful applied in the field of catalysis, bio-mimicking, molecular recognition, environmental analysis, biosensing and protein crystallization (Yang et al., 2019). MIPs have managed to remove many analytes such as dyes, oestrogens, pesticides, and personal care products (Cantarella et al., 2019). They also have been used in solid-phase extraction, organic synthesis, separation of enantiomers (Boulanour et al., 2017) and for sensors (Ahmad O.S et al., 2019). In literature, it has been reported that MIPs are a promising approach for the selective removal of trace metals in water (Cantarella et al., 2019). In this regard, they are referred as ion imprinted polymers (IIPs). Their high affinity and selectivity resemblance of that of natural receptors is very important if they are to be considered as an alternative receptor. Despite having many advantages, molecular imprinting is still riddled with challenges such as batch-to–batch variability, low long- term stability and skilled-labour intensive. This has caused scientists to use alternative methods which suppress these disadvantages (Ansari et al., 2019).

2.14 Performance comparison of different sorbents

Comparison of different sorbents that were used to adsorb pesticides in different matrices is shown in Table 2.6. It was observed that all the sorbents, except for MIP, had no selectivity. MIPs selectively rebind with the target molecule (s).

Table 2.6 : Adsorption capacity of different adsorbents in pesticides

Pesticide	Adsorbent	% Removal	Adsorbent capacity (mg g^{-1})	Selectivity	Reference
Fenuron	Carbon Nanotubes	90	0.0598-0.06918	-	Ali et al., 2019
Metribuzin	Pieurotus mutilus	70	3.3	-	Behloul et al., 2017
Pyraclostrobin	Mesoporous activated carbon	> 80	66.2	-	Suo et al., 2019
Methyl parathion	Watermelon peels	99	-	-	Memon et al., 2008
Carbofuran	Carbonaceous	70-80	212-208	-	Vinod et al., 2006
Carbofuran	MIP-MWTs	89.0 ± 4.8 to 93.6 ± 3.2	106.2	Yes	Zhang et al., 2019
Phenoxy carboxylic acid	Mag-MWCNTs-MIPs	86.7 -95.2	-	Yes	Yuan et al., 2020
Alachlor	biomass based-activated carbon	66-64	-	-	Ahmad et al., 2018

Ethaboxam	Juglans regia-derived activated carbon	89 and 99	-	-	Ahmad et al., 2018
Acetampirid	activated carbon from lignocellulosic agricultural waste	40.275	35.7	-	Mohammad et al., 2020
Imidacloprid and acetamiprid	MIPs	95.8-94.8	33.01 and 25.62	Yes	Chen et al., 2020
Chlorpyriphos Malathion Disulfoton Pirimiphos	Magnetic restricted-access carbon nanotubes (M-RACNTs)	-	0.55	-	do Lago et al., 2020

-means the information was not available

Paper I

This paper ‘challenges of template bleeding in molecularly imprinted polymer of pesticides,’ reviews the challenges encountered during template removal in MIP synthesis. It pays attention to the effect of washing techniques, pairing agents, and number of cycles needed for the template removal. It also reviews the use of dummy templating.

Challenges of template bleeding in molecularly imprinted polymer of pesticides

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Abstract

Molecularly imprinted polymers (MIPs) are material that exhibit selective recognition towards the desired template. It possesses advantages of high selectivity, low-cost and low limit of detection. It is a promising alternative for natural antibodies that suffer from poor stability and high cost. However, the MIP has challenges of expensive templates, the template might not get removed even after extensive washing or the MIP may be damage during the removal process and this step often requires lengthy optimization whereby can seriously alter the accuracy of the results. In this review we summarise the effective way of washing off template, paying attention to the effect of washing techniques, pairing agents, and number of elution cycles required for satisfactory template removal. It also reviews the use of dummy template and computational chemistry to select appropriate solvent and functional monomer.

Keywords: Template, elution, dummy-templating, template bleeding, thermally unstable compounds, molecularly imprinted polymers

Abbreviation

MIP-molecularly imprinted polymers

NIP-non-molecularly imprinted polymers

PHWE-pressurized hot water extraction

ASE-Accelerated solvent extraction

SPE-solid phase extraction

2.15.1 Introduction

Molecular imprinting has gained popularity in research since developed 50 years ago (Piletsky et al., 2020). It has been applied in several fields including SPE sorbents, sensing elements (Tan et al., 2020), potential clean-up systems (Wu et al., 2016) and chemical and food sciences (Dinc et al., 2019). MIPs are widely applied due to being stable, resistant to a wide range of pH, solvents, and temperature (Martín-Esteban et al., 2016) and cheap thus making them an alternative to the use of natural receptors. MIPs are cross-linked synthetic polymers that are obtained by polymerisation of monomer with the cross-linker in the presence of a template (target molecule) (Díaz-Álvarez et al., 2019). When the polymer has formed, a template is removed from the polymer using an organic solvent with a pairing agent, as a result leaves a material with binding sites that are complementary to the template in terms of shape, size, spatial arrangement, and chemical functionality to that of a template. MIPs can specifically rebind with the target analyte in the presence of closely related compounds.

Even though making MIPs requires minimal effort, molecular imprinting technology has short comings and challenges. The challenges are expensive templates, and the template might not get removed even after extensive washing or the MIP may get damaged during the removal process and this step often requires lengthy optimization which can alter the accuracy of the results (Liu et al., 2020). Another challenge is that the resulted cavities may not be selective and the signal between MIP and NIP may not be significant (Gonçalves 2020). MIPs also suffer from low adsorption capacity (Wan et al., 2021). Hence the use of structural analogue called a dummy template to imprint as to minimize template bleeding and to avoid high cost of the target analyte.

This paper reviews the effective ways of template washing, paying attention to the effect of washing techniques, pairing agents, and number of cycles has on the template removal. It also reviews green extraction solvents used to remove template after synthesis of molecularly imprinted polymers. A review on template bleeding was published previously (Lorenzo et al., 2011) which reviewed the effect of extraction technique on the removal of the template covering physically assisted extraction, solvent extraction and subcritical or supercritical solvents. Martín-Esteban et al., 2016 also published a review with emphasis solutions on how to minimise template bleeding. There are few reviews that solely outlines the template bleeding. Therefore, to the best of our knowledge this is the first review to outline the use of green solvents in template washing. This paper will help to address challenges that researchers face, or it will enable them to make informative decision on how to effectively wash the template without damaging the cavities and using solvents that are environmentally friendly.

2.15.2 Template bleeding / template leaching

There is a setback associated with MIPs which is called template bleeding. It is an incomplete removal of the template from the polymer network. This setback is persistent even after exhaustive washing steps, and later tends to interfere with the analysis especially trace analysis (Tang et al., 2019). This phenomenon results in less accessible recognition sites and lower binding kinetics (Zhang et al., 2017). This often results in doubt in quantitative accuracy and unreliability for trace level detection (Sun et al., 2019). The template is eluted using different washing techniques such solvent extraction, subcritical or supercritical solvents and physically assisted extraction. Pesticides are mostly washed by solvent extraction technique as observed in Table 1. Researchers might be mostly using this technique due to its simplicity and it does not require high temperatures which might damage the MIP. Accelerated solvent extraction (ASE) and continuous Soxhlet extraction had impressive template extraction efficiencies up to 94.2% and 99% but uses harsh chemicals that could potentially damage the environment, hence the use of pressurized hot water extraction (PHWE) as a feasible green extraction method that utilizes only water at elevated temperature and pressure conditions to remove template (Batlokwa et al., 2011). Water is not suitable for extraction of non-polar or organic compound hence the temperature is elevated in temperatures between 100^{o}C and 374^{o}C to decrease its viscosity, surface tension while diffusivity increases, water can effectively extract wide range of analytes with different polarities (e.g., Polychlorinated biphenyl and pesticides) from the environment (Gonçalves et al., 2017). Most pesticides are extracted from the environment using Soxhlet extraction, hence there is no information of pesticides extraction using pressurized hot water extraction in MIPs. PHWE was evaluated by application to three distinctly coloured MIPs for chlorophyll (green), quercetin (yellow) and phthalocyanine (dark blue) with subsequent monitoring of template removal and template bleeding by an ultraviolet spectrophotometer (Batlokwa et al., 2010). The template for determination of aromatic was eluted from the polymer using pressurized hot water extraction (Tavengwa et al., 2017)

Templates are removed using different solvents with a pairing agent. A pairing agent is an acid where mostly 10% is added. An acid should be carefully selected as the type of acid effects the removal of the template. Strong acid tends to be harsh and is associated with the massive loss of the polymer due to partial hydrolyses and the bleeding tends to decrease with the strength or size of the acid employed (Ellewanger et al., (2001). An acid is added to overcome the strong interactions between analyte and the MIP (Sanagi et al., 2013). Most of pesticides templates are washed off using methanol and acetic acid as shown in Table 2.7. Most of pesticides are highly soluble in methanol therefore able to dissolve the template off from the polymer while the acid interrupts the monomer-template bond formed during polymerisation. The NIP must be washed as Differences in the washing procedure have been shown to lead to unrealistically high selectivity factors of the MIPs, particularly in the case of swellable networks.

Table 2.7: Template washing techniques

Template	Extraction technique	Solvent	Reference
Methyl parathion	Solvent extraction	Methanol/water mixture (4/1, %v/v) containing 0.2*g* NaOH	Wang et al., 2019
Cyanazine	Solvent extraction	0.01 M calcium chloride ($CaC1_2$)	Saylan et al., 2017
Nicotinamide	Soxhlet extraction	Methanol/acetic acid (9:1, v/v)	Chen et al., 2020
Carbofuran and profenofos	Solvent extraction	Methanol and acetic acid (9:1 %v/v)	Sroysee et al., 2019
Carbofuran	Solvent extraction	Methanol and acetic acid	Zhang et al., 2018
Ethyl paraoxon	Solvent extraction	Methanol/acetic acid solution (9:1, v/v)	Wei et al., 2018
Phosmet	Soxhlet extraction	Ethyl acetate/methanol/acetic acid solution (4:4:2, v/v/v) for 48 h, then with methanol/acetic acid (8:2, v/v) for 24 h	Aftim et al., 2017
Imidacloprid	Soxhlet extraction	MeOH/acetic acid mixture (95/5, v/v)	E l-Akaad et al., 2020
Chlorpyrifos	Soxhlet extraction	Methanol/acetic acid (8:2 v/v)	Kumar et al., 2019
Pendimethalin	Soxhlet extraction	methanol and acetic acid (95:5, v/v)	Shahnazi et al., 2020

2.15.3 Effects of template bleeding

Persistent leakage of template during SPE procedure can affect the accuracy and precision of quantitative analysis (Sun et al., 2019). It also gives false results during on-line measurement in GC analysis when applied as a stationary phase in LC columns (Iskierko et al., 2017). This could lead to over estimation of the quantity of such compounds in real samples (Madikizela et al., 2018). It also increases limit of quantification (Ellwanger et al., 2001). Template bleeding also limits the application using any template especially trace analysis as it might elevate the sample background resulting in unreliable results (Hijazi et al., 2020; Zhao et al., 2020; Wei et al., 2018).

Therefore, template bleeding must be eradicated or minimized. The method that are used to minimise template bleeding includes the use of dummy template, more washings and selection of appropriate reagents for adequate washing.

2.15.3 Methods of minimizing template bleeding

2.15.3.1 Exhaustive washing

Template is removed from polymer network using different washing techniques with different solvents. Template removal step remains a problem as Some studies have reported that a small portion of template was not extracted even after extensive washing like it was shown by Figure 2.12. Pressurized hot water extraction (PHWE) was able to extract 100% template. The lack of complete removal of the template with ultra-sonic and Soxhlet is attributed to the low solvent accessibility to the more highly cross-linked domains of these polymers. Most of the researcher's state that the template was completely removed, and the question remains on whether template was really removed completely or was it adequately removed for that certain analysis. So whenever using any washing technique one needs to think of not damaging the cavities with harsh temperatures and chemicals. Adequate removal (not over washing) of MIP is needed to avoid damaging the MIP cavities and to avoid incomplete removal of MIP as indicated in Figure 2.11.

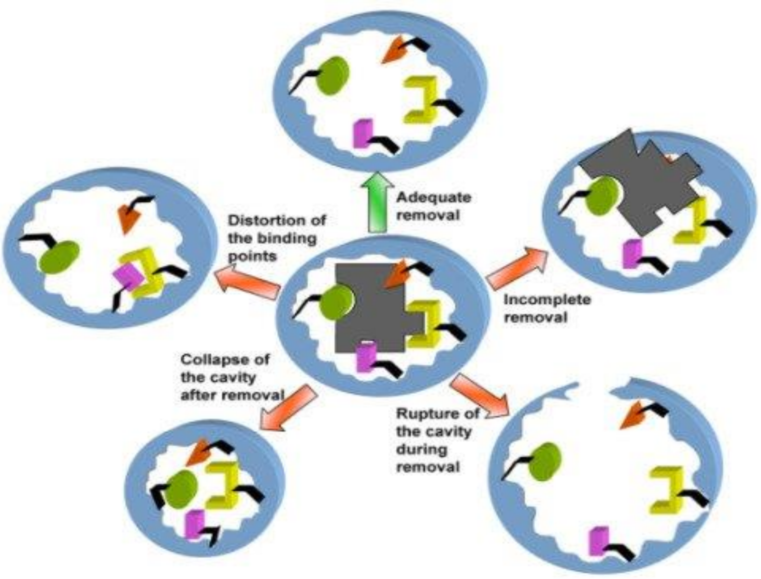

Figure 2.11: Processes that MIPs may undergo during washing processes

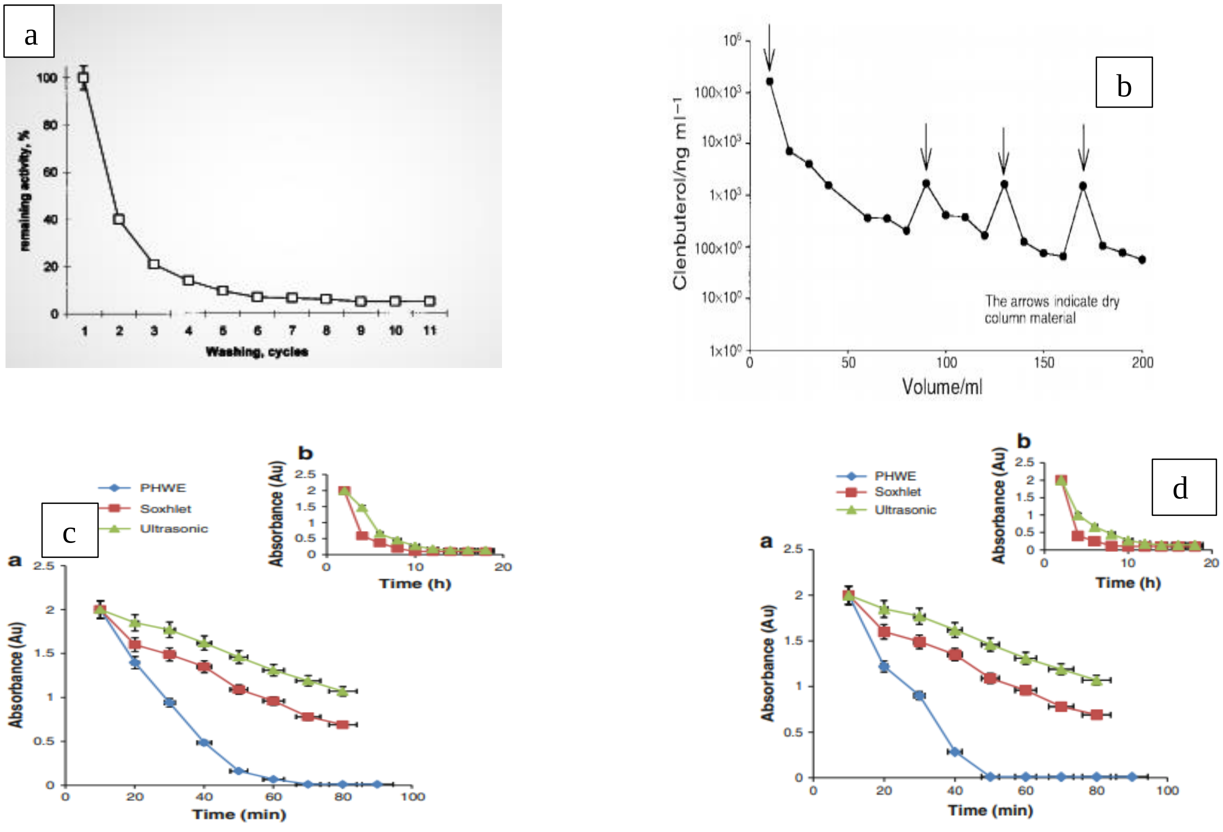

Figure 2.12: Template washing of (a) A HRP (b) elenbuterol (c) quercetin (d) phthalocyanine

2.15.3.2 Dummy templating

A dummy templating phenomenon came with Andersson in 1997 whereby a close structural analogue was used to substitute the real target molecule. The dummy template should be able to separate the two analogues as to not interfere with analysis (Xi et al., 2016). A dummy template resembles the target analyte in terms of size, shape and functionalities and it can be used to avoid template leakage as it does not interfere with analytical determination (Arabiet al., 2016). It can also be used when a template is unsafe, unstable (Zhao et al., 2018), low solubility and expensive (Marć et al., 2018). A dummy template (Nicotinamide) was used for removing imidacloprid and acetamiprid in tea polyphenols and nicotinamide was chosen as it has less toxicity and more accepted by consumers than the target analytes (Chen et al., 2020). Core–shell dummy molecularly imprinted polymer was used for recognition and extraction of organophosphorus pesticide (Wei et al., 2018). Dummy-surface molecularly imprinted polymers based on magnetic graphene oxide for selective extraction and quantification of pyrethroids pesticides in fruit juices (Zhao et al., 2020). An ionic liquid modified dummy molecularly imprinted polymer as a solid-phase extraction material for the simultaneous determination of nine organochlorine pesticides in environmental and food samples (Gao et al., 2013) and Magnetic dummy-template molecularly imprinted polymers based on multi-walled carbon nanotubes for simultaneous selective extraction and analysis of phenoxy carboxylic acid herbicides in cereals (Yuan et al., 2020).

Choosing a dummy template is a crucial step for successful imprinting. For example, acrylamide can also act a monomer when used as a template and has strong linking due to double bond with the crosslinker and as a result results in adequate removing of template even after exhaustive washing (Bagheri et al., 2019). Structural related analogues, fragments and isotopes are used as dummy templates (Sun et al., 2019) (put of pesticides). Structural analogues are the mostly used as a dummy template as isotopes are expensive.

To choose a structural analogue, (song et al., 2017) had used computational simulation method to select a dummy template that was suitable for four phenothiazine and (Marć et al., 2018) also used computer modelling method for to select optimal structural analogue. Computer modelling it is an effective method it saves time, reduces the use of organic solvents and reagents. Dummy templating results in low recovery amount and its availability is limited especially for homolog compound simultaneous determination (Zhu et al., 2016). The recoveries when using the actual template is higher than the dummy template also selection of dummy template using computer simulation for pesticides has limited information.

2.15.4 Computational modelling

Computer simulation for predicting the suitable eluting solvent for pesticide has not been studied. Eluting solvents are normally chosen based on previous experience. Computational chemistry can be used to drastically reduce the experimental effort in the solvent selection. Thermodynamic model COSMO-RS (Conductor-like Screening Model for Realistic Solvation) has been used for centrifugal partition chromatography (Bezold et al., 2017). Also, quantum chemical calculations with the simulation tool COSMO-RS were carried out to predict the partition coefficient (log*P*) of (S)-(+)-carvone and terpinen-4-ol in various solvent–water systems and validated afterwards with experimental data. COSMO-RS results show good prediction accuracy for non-polar solvents such as n-hexane, ethyl acetate and n-heptane even in the presence of salts and glycerol in an aqueous medium and n-heptane was chosen for the extraction of (S)-(+)-carvone due to high log*P* value (Janoschek et al., 2018).

2.15.5 Strength of the functional monomer and template

Previous experience or chemical intuition has been long used to select an appropriate monomer. For example, methacrylic acid has been selected in many cases due to its vital role played in the molecular imprinting history (Karim et al., 2017). These experimental steps usually involve the use of significant amounts of chemicals and solvents and, consequently, time- and resource-consuming work. Hence the use of computational chemistry to cover the draw backs associated with previous experience and chemical intuition. The development of computer simulation came in handy as saves time and chemicals. The computational simulation dated back in 1999 by the Sergeyeva group where the MIP reagents were screened for proper selection. They have used HyperChem 3.0 to determine the suitable functional monomer to the template and then various software's had been used since then (Xi et al., 2016). Different software's that were used are represented by the table 2.8. These software's are used to obtain the binding energy between the template and the monomer by studying their intermolecular interactions such as van der Waals forces, electrostatic, hydrophobic, and dipole-dipole interactions in the pre-polymerization mixture. (Ganjavi et al., 2017: Bakas et al., 2013). The more the interaction, the higher stability, and this result in better imprinting efficiency (Fu et al., 2015). The monomers with the highest template-monomer interaction are selected. Density functional theory method is mostly used for pesticides as it can be observed in Table 2.8**.** Observation DFT, which has become very popular in recent years, enables novel molecules of theoretical interest to be accurately studied at a lower computational cost as compared to ab initio methods (Yu et al., 2019). You can screen a lot of monomers using computational chemistry than you can do in the lab. The stronger the interaction between the monomer and the template the more challenging it is to remove the template. That is a reason why a template ration to other reagents should take into consideration as adding a lot of template results in excess template after reaction.

Table 2.8: Table of comparison of different methods for monomer-template interactions

Software used	Template	Selected monomer(s)	Chosen monomer	Total interaction Energy	Reference
Density functional theory method at B3LYP with 6-31 G	3-methylindole	Thiophene	Phenylenediamine	-0.77	Yu et al., 2019
		3methylthiophene		-1.78	
		pyrrole		-4.71	
		o-PD		-13.45	
		furan		-1.00	
		o-aminophenol		-2.56	
DFT-calculations at the B3LYP level with the 6-31G+(d,p)	Chloramphenicol (CAP)	Acrylic acid methacrylic acid acrylamide methylmethacrylate	Methacrylic acid	-	Xie et al., 2020
SYBYL™	Acetamide	25 commonly used monomers	Itaconic acid	-33.31	Viveiros et al., 2017
Molecular docking combined with quantum chemical calculations	immunoglobulin G (IgG)	m-phenylenediamine dopamine 3,4-ethylenedioxythiophene	m-phenylenediamine	-	Boroznjak et al., 2017

Quantum mechanical (QM) calculations and MD	Furazolidone	11 monomers	2-acrylamido-2-methyl-1-propanesulfonic acid	26.854 to -71.943	Rabelo et al., 2020

2.15.6 Effect of isomerism on template removal

Chiral pesticides such as metalaxyl, triazole, imazalil, tebuconazole and alpha-cypermethrin exhibit some degree of stereoselectivity, they have dependent toxicity because of having different pharmacokinetics and pharmacodynamics (Petrie et al., 201). They have the same physico-chemical properties in achiral environment and sometimes their bioactivity exists in one enantiomer. For example, the fungicidal activity of banalaxyl is mainly due to its R-enantiomer (Zhao et al., 2019) and the (+)-enantiomer of metalaxyl was less active than its (-)-enantiomer (Zadra et al., 2002; Zhao et al., 2018). Fipronil has enantioselective degradation in sediments (Wang et al., 2019). Tebuconazole degraded enantioselectivity in the roots and shoots (Lv et al., 2017). Two enantiomers of benalaxyl with chiral centres are indicated by Figure 2.13.

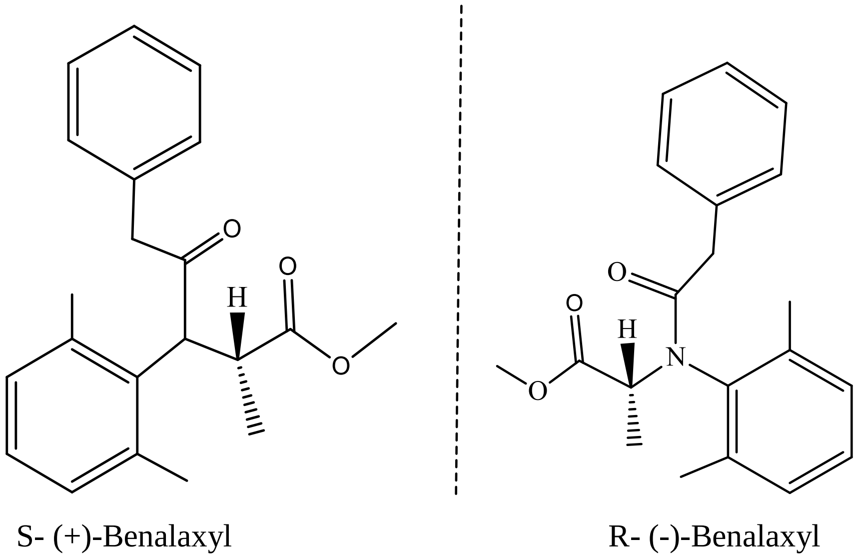

Figure 2.13: Enantiomers of benalaxyl pesticide

There has been report where pesticides are chiral imprinted (Zhang et al., 2017), where it has shown advantageous characteristics of improved stability towards mechanical and thermal stress, low-cost material and able to tolerate a broad range of solvents, bases, and acids (Rutkowska et al., 2018). However, these compounds are often treated as one compound rather than racemic mixtures whereby during washing procedure it is washed under assumption that it is not a chiral compound. For example, triadimefon is a chiral pesticides, during washing it was eluted as a whole compound rather than racemic mixture, it was washed with HAc-MeOH (10:90, v/v) to remove template residue until no TDF could be detected by LC–MS/MS (Zhao et al., 2017) and profenos MIP was Soxhlet-extracted with methanol and acetic acid (4:1, v/v) solution for 48 h and then Soxhlet-extracted with methanol for 24 h to fully elute the Profenos (Chen et al., 2020). This might result in incomplete washing as the solvent might be suitable for only one enantiomer. so therefore, they need to be handled as individual enantiomer.

2.15.7 Effects of unstable compounds

Extraction techniques such as heat reflux/Soxhlet, microwave/ultrasound assisted extraction are not suitable for template removals as they involve prolonged heating whereby thermally unstable compound maybe be lost (Tong et al., 2019) or degrade. MIPs cannot be exposed to very high temperature for fast template removal using these processes or other energy intensive extraction approaches (Kalogiouri et al., 2020). It is not suitable for thermosensitive templates hence the use of dummy template (Zhao et al., 2020). Even though Soxhlet extraction considered as being harsh on MIP, there are reports whereby this method has been used to remove traces of pesticides template from the MIP (Sanagi et al., 2013; He et al., 2015; Chen et al., 2020). The template removal process may be dependent on the template, as some pesticides compounds are not sensitive to heat.

Conclusion

The challenges and method of minimising template bleeding has been discussed. Template bleeding remain a challenge as there are minimal information on selection of appropriate washing reagents and washing techniques. There are efforts that are done to minimise template bleeding. The method includes the use of dummy template, the structural analogue that is like analyte in terms of size, shape, and functionalities it is mostly utilised as it does not interfere with analytical determination. more washing, the use of computational chemistry to simulate a suitable eluting solvent can be a great help as it minimises the use of lots of solvents. The monomer-template strength prediction is important as it helps with the decision of template washing based on the interaction information.

Chapter 3

3 Research objectives

This chapter addresses how This chapter provide list of materials and how the experiments were carried out.

3.1 Chemicals and reagents

For chemicals and reagents refer to chapter 4 in **paper II and III.**

3.2 General approach

- To synthesized MIP as outlined in the methodology of the respective papers.
- Characterisation of the material to gain the morphology and stability of the resulted MIP.
- Optimization was carried out where parameters such as sample pH, mass of the adsorbent, type of eluting solvent and eluting volume were investigated.
- The developed method was then applied to real samples to test their applicability from parameters such as EF and % RSD.

Figure 3.1 shows a general flow chart of the order of experiment for **paper II and III**. The sorbent material was first synthesized using polymerising reagents. and the resulted MIP was characterised to gain its physical properties.

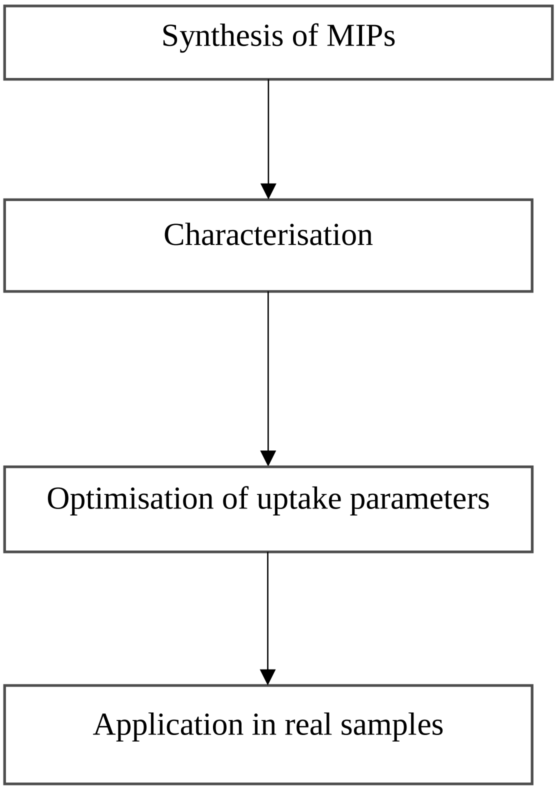

Figure 3.1: General scheme of the research approach

Chapter 4 and 5

Results and discussion

These chapters consist of paper II and III. In paper II a dummy template molecularly imprinted polymers which was synthesized using carbaryl as template was used to extract carbofuran from grapes. In Paper III a molecularly imprinted polymer was used as a sorbent to extract pirimicarb from orange sample.

Chapter 4

Paper II

This paper ‘’Determination of carbofuran by carbaryl dummy template molecularly imprinted polymer from grapes,” investigates the use of dummy template (carbaryl) which is a structural analogue to extract carbofuran from grapes.

Determination of carbofuran by carbaryl dummy template molecularly imprinted polymers from grapes

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Abstract

Carbofuran is one of the most toxic carbamate pesticides and it is banned in most countries. Carbofuran was determined by carbaryl dummy template molecularly imprinted polymer from grapes. FTIR characterization showed the C=O stretching absorbance in the region of 1714-1730 cm^{-1} between unwashed MIP, NIP, and washed MIP with different intensities. Various parameters affecting the extraction efficiency of the imprinted polymers were evaluated to optimize the selective preconcentration of carbamates in grape sample. Under optimal condition, the enrichment factor of up to 214.85 was obtained. The results during application showed that the grape sample 1 until 3 was found to contain no carbamates. This method was successfully applied to quantitively detect carbamates residue in grape sample.

Keywords: Dummy template, molecularly imprinted polymer, pre-concentration, selectivity, carbamates

4.1 Introduction

Carbofuran (2, 3-dihydro-2, 2-dimethylbenzofuran-7-yl methylcarbamate) is one of the most toxic broad-spectrum carbamates (Mishra et al., 2020). It is applied in potatoes, corn and soyabeans (Jirasirichote et al., 2017). It possesses high mobility and solubility, it often runoff from treated sites contributing to contamination of groundwater (Vithanage et al., 2016). It is very toxic to mammals, and exposure to it can result in blurred vision, coma, nausea, weakness, and abdominal cramping (Vimal et al., 2019). It is also known to inhibit cholinesterase enzyme, which results or lead in neural impairment (Gupta et al., 2019). For safety of people, China set the maximum residue limit of 0.2 $mg\ kg^{-1}$ in grains and oilseeds, and United States Environmental Protection Agency (EPA) set at 0.1 $mg\ kg^{-1}$ in green bean, banana, coffee and rice (Zhang et al 2017). Its toxicity has resulted in it to be banned in United States and Europe. However, due to its high effectiveness and low cost, it is still used in country like Brazil (Campos-Garcia et al., 2016). Even though is banned in other countries it is still detected in the environment. Thus, it is necessary to find a method that can detect it at a low concentration.

Several techniques have been developed or applied for determination of carbamates in different environmental samples, including food supplements, juices, and liver (Moreno-González et al., 2016; Moreno-González et al., 2015; Yeneva etal., 2019). Carbamates occur in multi-composition in real samples like ground water and agricultural soils, so they have complex matrix and occur at low concentration, therefore there is a need for sample clean-up prior analysis. Hence the use of molecularly imprinted polymers. MIPs are materials that have complimentary design in terms of shape and functional groups of that of a template or a target compound. MIPs selectively and specifically bind with the similar molecule or the target molecule from the closely related analytes (Dinc et al., 2019; Ansari and Mosoum, 2019; Cantarella et al., 2019). MIPs are commonly used as a sorbent because of it having great chemical stability and selectivity (Udomsap et al., 2018), recognition specificity, structure predictability (Sajini et al., 2019), physical robustness (Zhang et al., 2019), easily availability (Yang et al., 2019) and a chance of not doing extensive pre-treatment when analysing complex matrices (Guoning et al., 2019). However, the removal of the template remains difficult during MIP synthesis.

The inadequate removal of template results in template bleeding that can interfere with the accuracy and reliability for trace level detection, hence the use of a dummy templates. A dummy template can be used when it is difficult to synthesize with a chosen template, when the template is not being able to dissolve in a common porogen (MarĆ et al., 2018) and on highly poisonous compounds (Arak et al., 2019). A dummy template is a compound that has similar physico-chemical properties to that of a desired template (Tahir et al., 2012) and has a similar structure to that of an analyte (Tang et al., 2019) in terms of shape, size, and functionalities without interfering with analytical determination (Arabi et al., 2016). Structural and isotopic template are used as dummy molecules and they show excellent imprinting efficiency. Isotopic dummy templates are not mostly used because they are costly and they are not easily accessible (Sun et al., 2019).

There have been a lot of articles reporting the excellent recognition abilities for the pesticides by the dummy templates (Wei et al., 2018; Zhao et al., 2020; Chen et al., 2020; Sun et al., 2019: Zhang et al., 2020). In the present work, carbaryl has been used to imprint a dummy template for the determination of carbofuran because it exists in complex matrices. The experiments have been carried out using miniaturised pipette-tip extraction whereby a small sorbent was used. To the best of our knowledge this is the first paper to use dummy template for the detection of carbaryl using pipette-tip miniaturised solid phase extraction.

4.2 Materials and methods

4.2.1 Chemicals and reagents

Carbaryl, carbofuran, propoxur, pirimicarb, methacrylic acid, ethylene glycol dimethacrylate (EGDMA) was purchased from Sigma-Aldrich (Johannesburg, South Africa). Azoisobutyrobutyronitrile (AIBN), methanol, acetic acid and acetonitrile were also supplied by sigma-Aldrich (Johannesburg, south Africa). HPLC grade acetonitrile (ACN) and methanol (MeOH) were supplied by Merck (Johannesburg, South Africa). Sodium hydroxide and nitric acid were also obtained from sigma Aldrich (Johannesburg, South Africa).

4.2.2 Preparation of stock solutions and real solutions

A 1000 mg L^{-1} stock solution of carbofuran was prepared in methanol and subsequent dilutions using water were made for working standards. For real sample analysis, 1000 mg L^{-1} of each carbaryl, propoxur, pirimicarb and carbofuran were made using acetonitrile HPLC grade acetonitrile and working standards were diluted using HPLC grade acetonitrile.

4.2.3 Instruments

The functional group for the polymer was analysed by using alpha Fourier transform infrared spectrometer (FTIR) instrument (Johannesburg, South Africa). Ultraviolet analyses of carbofuran were recorded using a Shimadzu Ultraviolet-Visible (UV-vis) with maximum wavelength was 288 nm. LCMS-9030 Ultra High-performance Liquid Chromatography-Quadrupole Time-of-Flight mass spectrometry (Shimadzu, Japan) equipped with Shim-pack Velox separatory column (100mm×2.1mm, with particle size of 2.7 µm, Shimadzu, USA) and SYNAPT GI Q-TOF high-definition mass spectrometry (Shimadzu, Japan) was used for real sample. Samples were analysed using linear gradient elution. The flow rate was set at 0.4 mL/min and the injection volume was 6µL. MS was operated in positive ion mode with MRM detection. The quantification of 4 carbamates was performed.

4.2.4 Synthesis of D-MIPs and NIP

The method was adapted and modified from Atayatet et al (2019). The D-MIP was synthesized by precipitation polymerisation technique. During the synthesis of carbofuran D-MIP, dummy template (carbaryl) (0.1 mmol) was dissolved in 50 mL porogen solvent acetonitrile in a 250 mL round bottom flask. MAA (0.3376 mL), EDGMA (0.37755 mL) and initiator AIBN (40 mg) were also added to the flask and the solution was taken under nitrogen gas flow for 10 min and the flask, was closed and sealed and placed in a water bath in a sonicator at 80ºC for 3 hours. The resulted MIPs were filtered, air dried for 24 h and then ground carbaryl (template) was then eluted from the polymers with MeOH and acetic acid of ratio of 9:1 v/v until the signal at UV-VIS spectrometer at 288 nm was zero. The polymer was then air dried for 24 h. The reference material NIP was prepared by using the conditions defined above for MIP-synthesis except for the addition of the dummy template molecule (carbaryl).

4.2.5 Miniaturised pipette-tip preparation

A modified method from Seldi et al (2019) and Tavengwa et al (2016) was used miniasturised pipette-tip extraction. A polypropylene micropipette was used where microscale amount of a sorbent was packed inside a pipette tip. The MIP sorbent was packed between glass wool and conditioning step was applied using a suitable solvent to wet or activate the sorbent functional group and ensure consistent interactions between analytes and sorbent. By replicated aspirating /dispensing cycles, the extraction procedure would be reached to equilibrium Polypropylene pipette tips were. A total mass of ≈ 18 mg of the MIPs were loaded into a 1000 µL pipette tip which was pre–loaded with a fixed mass of 10 mg of glass wool. The pipette tips loaded with MIPs were then conditioned by aspirating and dispensing 1000 µL of 50% (v/v) water/methanol solution three times. A 1000µL aliquot of the solution with carbofuran compound was aspirated onto the D-MIPs and dispensed back into the same sample tube 20 times.

4.2.6 Real sample analyses

The Grape samples were cut into small pieces and then homogenized in a stainless-steel blender. Then, 50 *g* of homogenized sample was transferred to a 50-mL plastic centrifuge tube and centrifuged for 5 min at 3600 rpm. The supernatant was filtered and diluted in 100 mL volumetric flask and adjusted to pH of 3. For method validation, 5 ml of 0.5 and 0,25 mg L^{-1} of mixture of 4 standards (propoxur, carbaryl, pirimicarb and carbofuran) were sprayed evenly on the samples for 1 h and 50 ml of distilled water was added and ultrasonically extracted for 30 min and diluted to 100 mL.

Enrichment factor

Optimization parameters investigated were pH (3-11), mass (5-50 mg), aspirating/dispensing cycles (1-40), eluting solvent (MeOH, acetonitrile, chloroform, CCl4) eluting volume (200-1000) and loading solvent (200-100). For optimization of extraction conditions, enrichment factor (EF) was used to evaluate the extraction efficiency under different conditions. The enrichment factor was defined as the ratio between the analyte concentration (Ce) and the initial concentration of analyte (Co) within the sample:

$$EF = \frac{C_e}{C_o}$$

Ce was calculated by extrapolating the data from UV-VIS and LCMS standard curve.

4.3 Results and discussion

4.3.1Fourier transform infrared (FTIR)

Fourier transform infrared (FTIR) (Fig 4.1) was used to determine functional groups of washed and unwashed molecularly imprinted polymers, template (carbaryl) and NIP. It was performed to determine the functional groups of the MIP before and after washing. The decrease in intensity of washed MIP may indicated the success template leach out. The peak at 1714-1730 cm^{-1} on washed and washed MIP could be the presence of the template which is unsaturated easter and 1089 and 1085 cm^{-1} on unwashed and washed MIP is a result of aliphatic easter which is the template. The C=O stretching absorbance in the region of 1714-1730 cm^{-1} of NIP may be due to presence of excess polymerisation reagents (EDGMA, methacrylic acid) that were left after the synthesis.

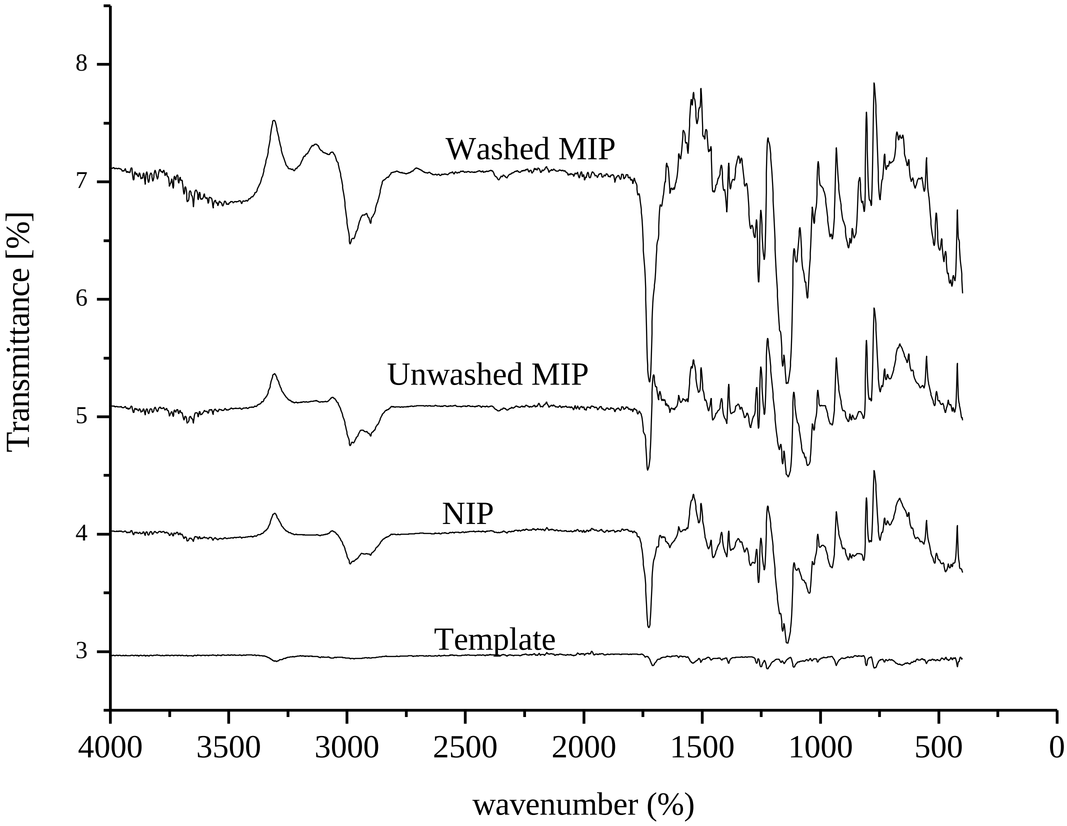

Figure 4.1: FTIR for functional group of NIP, washed and unwashed MIP

4.3.2 Effect of sample pH

The effect of sample pH is represented by Figure 4.2. It was performed by adjusting pH from 3 to 11. The amount adsorbed for template was changing with change in pH which means that the extraction of carbofuran was pH dependent. pH of 3 was optimum and used for subsequent experiment. The enrichment factor was high at pH 3 because in acidic condition the acid protonates the carbonyl group from carbaryl compound, and the resulted solution interact with the surface of the adsorbent via non-covalent interaction including van der Waal forces, hydrogen bond and hydrophobic bond between carbofuran and the active binding sites of MIP surface. The strong interactions result in high extraction recovery. The enrichment factor was low at pH 11 because carbamates are esters which are easily broken and easily hydrolysed in alkaline solution (Santalad et al., 2010; Gao et al., 2020) therefore results in low extraction recovery.

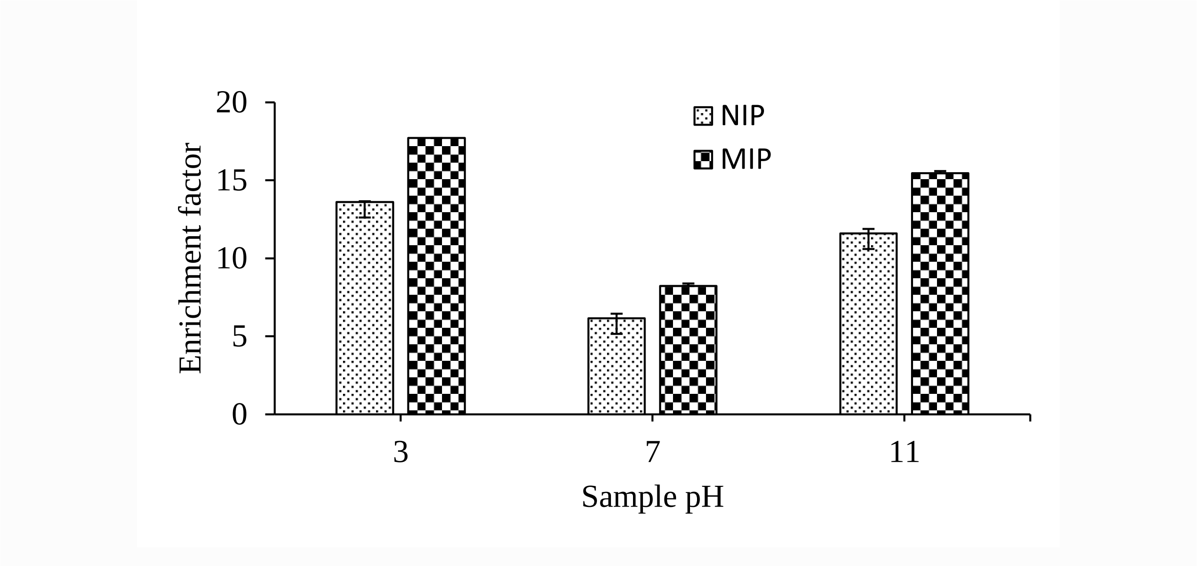

Figure 4.2: Effect of sample pH on the enrichment of carbofuran (n = 3, RSD). Experimental conditions; mass of glass wool = 18mg, mass of NIP/MIP = 10 mg, sample concentration = 0.5 mg L-1, aspirating/dispensing cycle = 20, eluting volume = 1000 µL, sample volume = 1000 µL, eluting solvent = methanol.

4.3.3 Effect of mass

The effect of mass is represented by Figure 4.3 The dosage of adsorbent is of vital significance that can directly affect adsorption efficiency of carbofuran. The experiments were done up to 50 mg because of the clogging of the pipette-tip. Mass of 50 mg was used for subsequent experiment as it gave optimum extraction recovery. The enrichment factor increased with increase in adsorbent dose. This is due to increase in availability of binding sites on the polymer that rebind with the carbofuran compound. When the amount of sorbent was small, there were few active sites to bind with carbaryl resulting in low enrichment factor. The similar trend was observed by Gao et al. (2020) where the extraction efficiency increased with increasing amount of adsorbent up, also Liu et al. (2018) obtained similar results in extraction of triazole pesticides where extraction efficiency of the triazole pesticides rapidly increases with increasing amount of M-MOF-199.

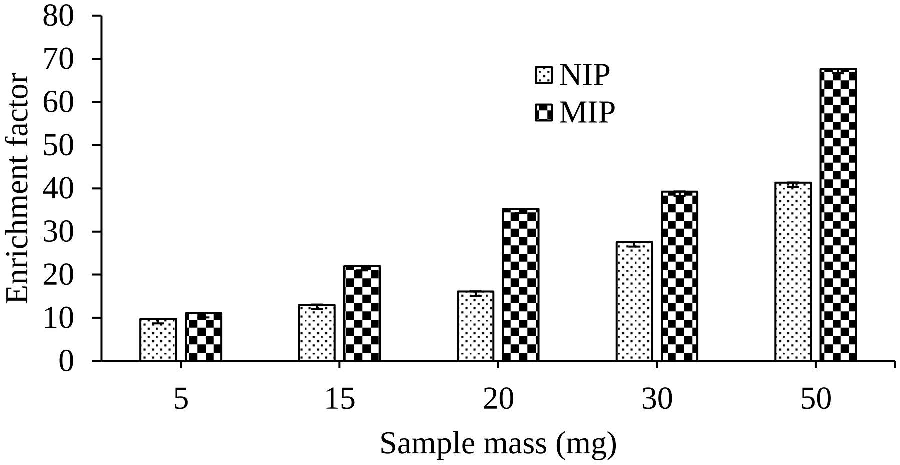

Figure 4.3: Effect of sample mass on the enrichment of carbofuran (n = 3, RSD). Experimental conditions mass of glass wool = 18 mg, pH = 3, sample concentration = 0.5 mg L-1, aspirating/dispensing cycle = 20, eluting volume = 1000 µL, sample volume = 1000 µL, eluting solvent = methanol.

4.3.4 Aspirating/ dispensing cycles

The effect of aspirating and dispensing cycles is represented by Figure 4.4. The number of aspirating/dispensing cycles is another important parameter that affects the adsorption efficiency of the extraction. Carbofuran had the highest enrichment factor at 10 cycles, and it was taken as the optimum value for the subsequent experiments. There was reduction of extraction efficiency of carbofuran at cycles greater than 10. This might be due to analyte being extracted from the sorbent eluate back into the solution causing low adsorption efficiency when the dispensing/aspirating cycles increases. The same trend was observed by Rezaei Kahkha et al (2017) in determination of diazon pesticide using modified multi-walled carbon nanotubes as a pipette-tip solid phase extraction sorbent.

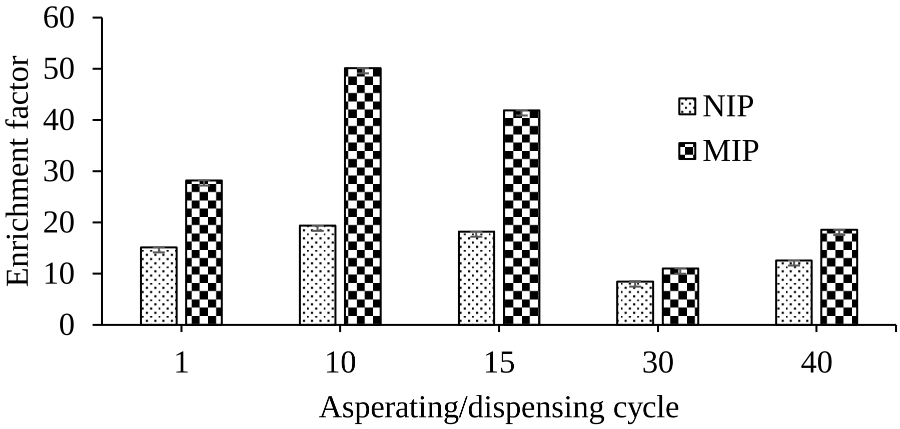

Figure 4.4: Effect of aspirating Effect of aspirating and dispensing cycles on the enrichment of carbofuran (n = 3, RSD**)**. Experimental conditions mass of glass wool = 18 mg, mass of adsorbent = 30 mg, pH = 3, sample concentration = 0.5 mg L^{-1}, eluting volume = 1000 µL, sample volume = 1000 µL, eluting solvent = methanol.

4.3.5 Effect of elution solvent

The type of eluting solvent played a huge role in extracting of carbofuran using a dummy template molecularly imprinted polymers. Methanol, acetonitrile, chloroform and carbon tetrachloride were choosen for the elution of carbofuran from D-MIP (Figure 4.5). Chloroform gave optimum recoveries and this may be because of its polarity. In order to have high extraction efficiency, the solvent should have high capability to disssolve the target analyte therefore chloroform may have polarity closer to that of carbofuran than other solvent. The analytes in chloroform solution may have had stronger hydrogen bond which may have enduced efficient eluting.

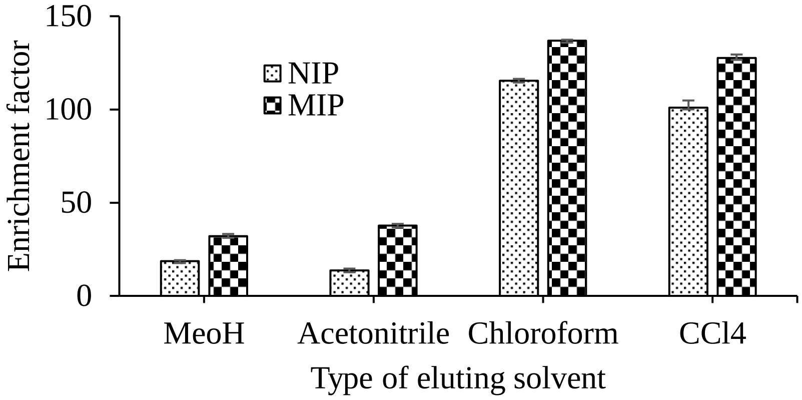

Figure 4.5: Effect of elution solvent on the enrichment of carbofuran (n = 3, RSD). Experimental conditions mass of glass wool = 18 mg, mass of adsorbent = 30 mg, aspirating/dispensing cycle = 10, pH = 3, sample concentration = 0.5 mg L^{-1}, eluting volume = 1000 µL, sample volume = 1000 µL, eluting solvent = methanol.

4.3.6 Effect of loading solvent

The effect of loading solvent was determined and represented by Figure 4.6. The extraction effeciency of the MIP increased with increase in loading volume as indicated by the gragh in Figure 4.6. This was due to the fact that there was a high amount of analyte in the solution to be extracted. The optimum loading volume was at 1000 µL. The low recovery on small loading volume may due to more active site of the polymer that result in less occupation from the analyte.

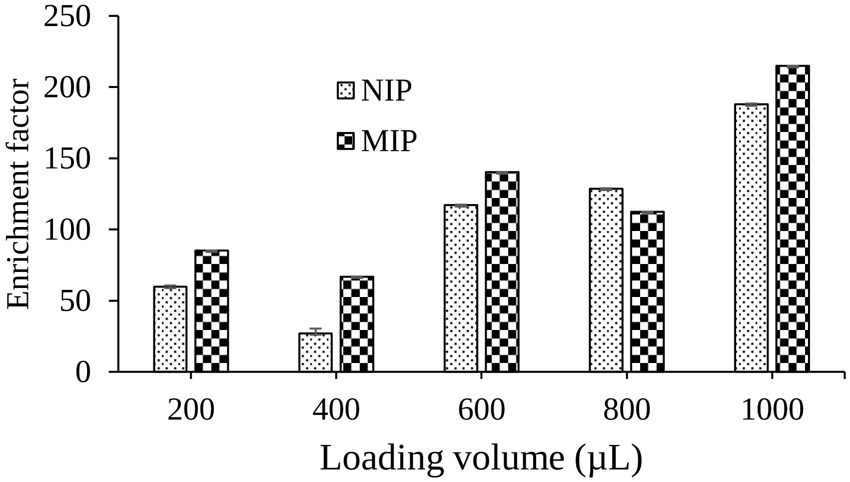

Figure 4.6: Effect of loading volume on the enrichment of carbofuran (n = 3, RSD). Experimental conditions mass of glass wool = 18mg, mass of adsorbent = 30mg, aspirating/dispensing cycle = 10, pH = 3, sample concentration = 0.5 mg L^{-1}, eluting volume = 1000 µL, sample volume = 1000 µL, eluting solvent = methanol

4.3.7 Effect of elution volume

The effect of elution volume is represented by Figure 4.7 below. The volume of 200-1000 µL was used in order to determine the optimum. The experimental results showed that the enrichment factor increased with an increase in elution volume as shown by Figure 7. This was a result of having adequate amount of elution volume to dissolve the analyte. And too small volume can results in incomplete elution while excessive amount of eluent can results in decrease in sensitivity leading to low extraction efficiency. So therefore adequate amount of eluting solvent is required.

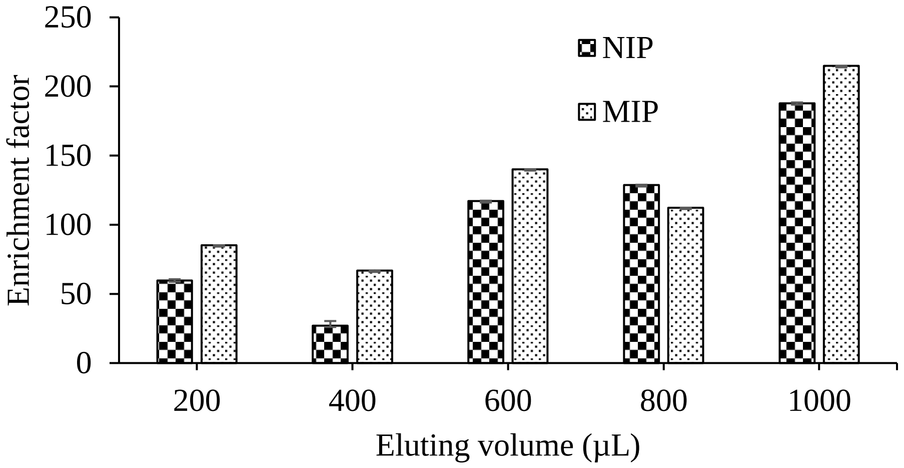

Figure 4.7: Effect of eluting volume on the enrichment of carbofuran (n = 3, RSD). Experimental conditions mass of glass wool = 18mg, mass of adsorbent = 30mg, aspirating/dispensing cycle = 10, pH = 3, sample concentration = 0.5 mg L^{-1}, eluting volume = 1000 µL, sample volume = 1000 µL, eluting solvent = methanol.

4.3.8 Real sample analysis

The potential of the developed MIP-SPE method for the selective sample clean-up were investigated in the analysis of grapes. The fruit samples were extracted by using the optimized MIP-SPE procedure. It was found that none of the analytes were not detected in the grape samples. This was probably because the carbamates were absent or present at ultra-trace levels in the fruit samples.

Conclusion

In the present study, a pipette-miniaturised solid phase extraction was utilised whereby a dummy template (carbaryl) MIP was used to extract four carbamates (carbaryl, pirimicarb, propoxur and carbofuran). The study demonstrated that MIP placed in the pipette-tip were effective for simultaneous determination of carbamates. The method was pH dependent it changed with change in pH. The investigation revealed the optimum mass of 50 mg with 10 dispensing/aspirating cycles. Chloroform showed the highest enrichment factor. It was found that none of the analytes were detected in the grape samples. This was probably because the carbamates were absent or present at ultra-trace levels in the fruit samples.

Chapter 5

Paper III

This paper ‘Solid phase extraction of pirimicarb from oranges using molecularly imprinted polymers as sorptive materials’ uses MIP as a sorbent to extract pirimicarb at low concentration.

Solid phase extraction of pirimicarb from oranges using molecularly imprinted polymers as sorptive materials

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Abstract

pirimicarb is a carbamate pesticide used for controlling pests on vegetables and is used in gardens and household. Pirimicarb was determined using molecularly imprinted polymer from oranges. The MIP material was characterised with FTIR where all the peaks were similar except their intensities. The MIP and NIP were optimised using different parameters and enrichment factor of 19 was obtained. The linear range was 0.25-2.5 mg L^{-1} and percentage recoveries of three carbamates ranges were 69.43 -84.67%. The limit of detection and limit of quantification 0.41-0.61 and 1.24-1.86 mgL^{-1}. The method indicated repeatability as the RSD ranged from 1.37-9.23. The potential of the developed MIP-SPE method for the selective sample clean-up were investigated in the analysis of oranges. It was found that none of the analytes were detected in the orange samples.

Keywords: Solid phase extraction, molecularly imprinted polymers, sorptive material, selectivity, pre-concentration

5.1 Introduction

Pirimicarb ([2-(dimethylamino)-5,6-dimethylpyrimidin-4-yl] N, N-dimethylcarbamate) is a carbamate pesticide used for controlling pests on vegetables (Zulfigar et al., 2019), selective aphicide in agriculture (Andrews et al., 2004; Champagne at al., 2019) and gardens and household use (Cakir et al., 2019). It also acts as insecticide against aphids in vegetables and fruits (He et al., 2018). Pirimicarb is moderately toxic (Ilktaç et al., 2020) but Due to excessive use they can leach to the ecosystem (Demir et al., 2020) causing environmental contamination and result in human health issues relating to cancer (Selva et al., 2017). This led to regulatory organisation limiting residual levels of pirimicarb in different matrices. Brazilian laws had 0.05 to 1 mg kg^{-1}, depending on the type of crop (Selva et al., 2017) and World Health Organization (WHO) have recommended tolerable daily intake (TDI) of

0~0.02 mg kg−1(Zulfigar et al., 2019). So therefore, it is important to identify and remove pirimicarb from the environment.

Several techniques have been developed or applied for determination of carbamates in different environmental samples such as water samples (Donato et al., 2017; Tsai et al., 2020). The techniques that were used lack selectivity hence the use of molecularly imprinted polymers (MIPs). MIPs are materials that are formed by copolymerisation of functional monomer with crosslinking monomer in the presence of template, the template is later removed to have a material that have cavities that are complimentary in terms of shape, size, and functional groups to that of target molecule (Chen et al., 2016; Bagheri et al., 2019; Azizi et al., 2020). The success of the imprinting depends on the selection of pre-polymerisation reagents (Kalogiouri et al., 2020).

There have a lot of articles reporting excellent recognition abilities of MIPs using pirimicarb as a template (Cakir et al., 2019; Ilktaç et al., 2020; Sun et al., 2006; Gao et al., 2008). In the present work, solid phase extraction has been used in determination of pirimicarb oranges. The experiments have been done using MIPs as a sorbent that was synthesized using precipitation polymerisation technique.

5.2 Materials and methods

5.2.1 Chemicals and reagents

Carbaryl, carbofuran, propoxur, pirimicarb, methacrylic acid, ethylene glycol dimethacrylate (EGDMA) were purchased from Sigma-Aldrich (Johannesburg, South Africa). Azoisobutyrobutyronitrile (AIBN), methanol, acetic acid and acetonitrile were also supplied by sigma-Aldrich (Johannesburg, south Africa). HPLC grade acetonitrile (ACN) and methanol (MeOH) were supplied by Merck (Johannesburg, South Africa).

5.2.2 Preparation of stock solutions and real solutions

A 1000 mg L^{-1} stock solution of pirimicarb was prepared in methanol and subsequent dilutions using water were made for working standards. For real sample analysis, 600 mg L^{1} carbaryl and 1000 mg L^{-1} propoxur were made using acetonitrile HPLC grade acetonitrile and working standards were diluted using HPLC grade acetonitrile.

5.2.3 Instruments

The functional groups for the polymers were analysed using alpha Fourier transform infrared spectrometer (FTIR) instrument (Johannesburg, South Africa). Ultraviolet analyses of pirimicarb were recorded using a Shimadzu Ultraviolet-Visible (UV-vis) with maximum wavelength was 288 nm. LCMS-9030 Ultra High-performance Liquid Chromatography-Quadrupole Time-of-Flight mass spectrometry (Shimadzu, Japan) equipped with Shim-pack Velox separatory column (100mm×2.1mm, with particle size of 2.7 µm, Shimadzu, USA) and SYNAPT GI Q-TOF high-definition mass spectrometry (Shimadzu, Japan) was used for real sample. Samples were analysed using linear gradient elution. The flow rate was set at 0.4 mL/min and the injection volume was 6µL. MS was operated in positive ion mode with MRM detection. The quantification of 4 carbamates was performed.

5.2.4 Synthesis of MIP and NIP

This method was adopted from Sanagi et al. (2013). 0.5mmol template was dissolved in 70 mL acetonitrile solvent. 5 mmol of MAA were added to the round-bottom flask and stirred for 10 min. EDGMA (0.01 mol) cross-linker was added then purged for 2 min with nitrogen gas, and then the lid was closed. The round bottom flask was opened and 50 mg AIBN initiator was added. After adding, the polymerisation mixture was Purged again for 2 min with N_2 gas. The lid was then greased then closed and covered with parafilm and the mixture of the pre-polymerization reagents was heated in an oil bath at 80^{o}Cfor 3 h.

5.2.5 Real sample analyses

The method was adopted from song et al (2018) and modified. Grape samples were cut into small pieces and then homogenized in a stainless-steel blender. Then, 50.0 *g* of homogenized sample was transferred to a 50-mL plastic centrifuge tube. After high-speed centrifugation about 3 min at 9600 rpm, the supernatant was filtered. The filtered sample solution was transferred to a 100-mL volumetric flask and make up to volume with distilled water.

Enrichment factor

Optimization parameters investigated were sample pH (3-11), mass (20-100 mg), sample volume, loading solvent (1-5 mL) and sample concentration (0.5- 2.5). For optimization of extraction conditions, enrichment factor (EF) was used to evaluate the extraction efficiency under different conditions. The enrichment factor was defined as the ratio between the analyte concentration (Ce) and the initial concentration of analyte (Co) within the sample:

$$\mathrm{EF} = \frac{C_e}{C_0}$$

Ce was calculated by extrapolating the data from UV-VIS and LCMS standard curve.

5.3 Results and discussion

5.3.1 FTIR of pirimicarb molecularly imprinted polymer

Fourier transform infrared (FTIR) (Fig 5.1) was used to determine functional groups of washed and unwashed molecularly imprinted polymers, template (carbaryl) and NIP. It was performed to determine the functional groups of the MIP before and after washing. All the peaks in unwashed MIP, NIP and washed MIP almost had peaks in the same region but had different intensities. Unwashed MIP was more intense while NIP and washed MIP had no significant difference in their intensities, and this may be due to being subjected under same washing conditions. The decrease in intensity of washed MIP may indicated the success template leach out. The C=O stretching absorbance in the region of 1716, 1713 and 1719 cm^{-1} between unwashed MIP, NIP and washed MIP may be due to presence of excess polymerisation reagents (EDGMA and methacrylic acid) that were left after the synthesis, or the C=O bond that of polymethacrylate. The absorbance of washed MIP at 1714 cm^{-1} was more intense than the others followed by NIP. This was due to interruption of the non-covalent interaction (hydrogen bonding) between the template and the functional monomer (MAA) during template removal. Similar results were observed by Sanagi et al. (2013).

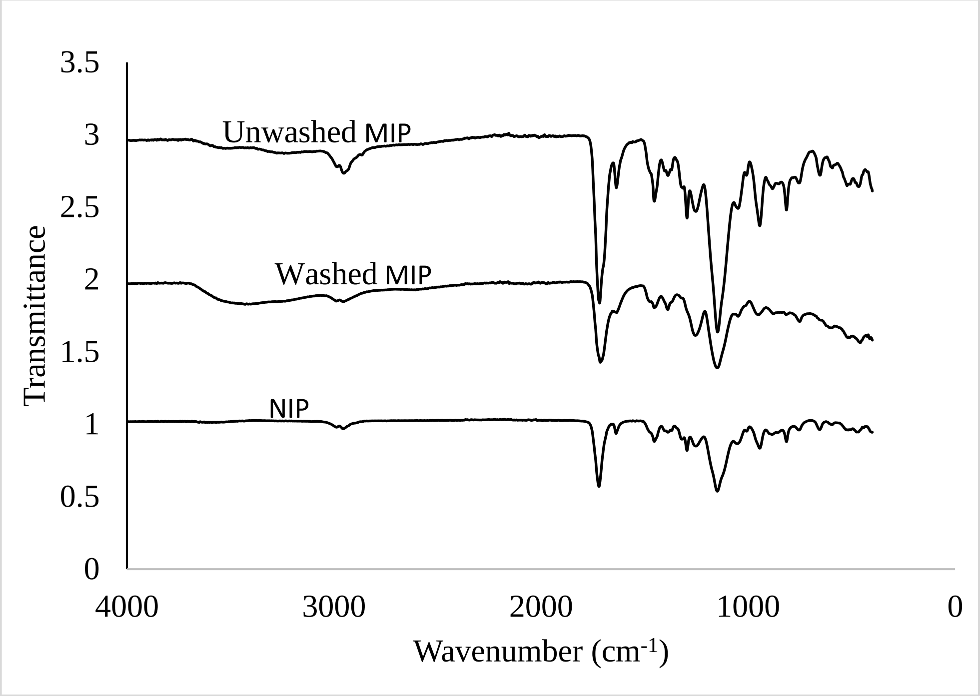

Figure 5.1: FTIR for functional group of NIP, Washed and unwashed MIP

5.3.2 Effect of sample pH

The effect of sample pH is represented by Figure 5.2. It was performed by increasing pH (3, 7 and 11). The extraction efficiency was optimum at pH 3. The amount adsorbed was changing with change in sample pH which meant that the extraction of carbofuran was pH dependent. The enrichment factor was low at pH 11 because carbamates are esters where they are easily broken and easily hydrolysed in alkaline solution (Sanatland et al., 2010; Gao et al., 2020). The higher recovery at pH 3 is due to protonated charged molecules that fit the binding sites of the polymer. The optimum results obtained agreed with the observations of Guo et al. (2020) where good extraction efficiencies for five carbamates were observed at pH 2 to 6 using graphene-oxide-based composite. Also, Gao et al. (2020) and Sereshti et al. (2020) obtained similar results in carbamates and pesticide using azamacrocycle-based adsorbent.

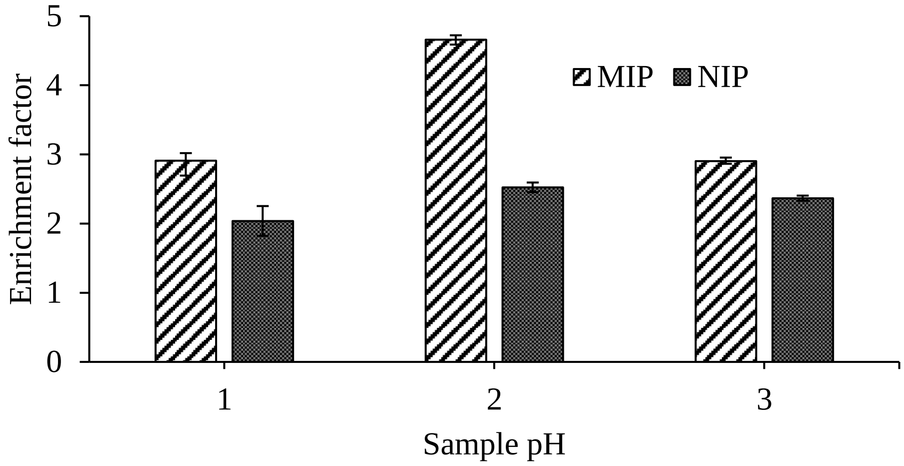

Figure 5.2: Effect sample of pH (n = 3, RSD). Experimental conditions: mass of glass wool = 30 mg, mass of NIP/MIP = 50 mg, sample concentration =1.0 mg L^{-1}, sample volume = 3 mL, eluting volume = 3 mL, eluting solvent = acetonitrile, washing solvent 2:3 v/v (MeOH/H_2O)

5.3.3 Effect of adsorbent mass

The effect of adsorbent mass is represented by Figure 5.3. It was performed by increasing mass from 20 until 100 mg. The extraction efficiency increased with mass dosage, indicating that 100 mg MIPs were sufficient for the extraction of pirimicarb using MIPs. The observed trend was due to MIPs having higher porosity, larger surface area and lots of binding sites resulting in high extraction efficiency. This trend was also observed by Ji et al. (2017) for chloroacetamide herbicides where the extraction efficiency increased with increase in mass until the equilibrium was reached at 50 mg. The NIP also followed a similar trend but with less enrichment factor due to less selectivity and minimal porosity.

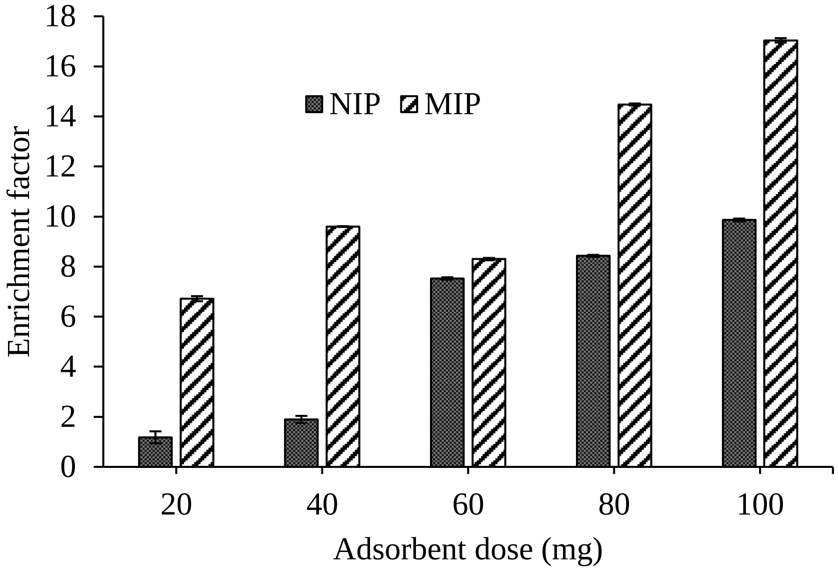

Figure 5.3: Effect of sample mass (n = 3, RSD). Experimental conditions mass of glass wool = 30 mg, sample pH = 3, sample concentration =1.0 mg L^{-1}, sample volume = 3 mL, eluting volume = 3 mL, eluting solvent = acetonitrile, washing solvent = 2:3 v/v ($MeOH/H_2O$)

5.3.4 Effect of sample volume

The sample volume is one of key factors to evaluate the extraction ability of sorbents for analytes at low concentrations. The effect of sample volume is represented by Figure 5.4. It was performed by increasing the sample volume from 0.5 to 5 mL. The extraction efficiency increased with increase in sample volume. A volume of 5 mL was used for subsequent experiments. The recovery of pirimicarb with 0.5 mL was small due to less interaction with active /binding sites of the extracting material. It kept on increasing due to high active binding sites that are not easily saturated. The NIP decreased at 3.5 mL probably because the active sites of the sorbent was saturated, and thus, additional analytes could not be adsorbed with increasing loading volume.

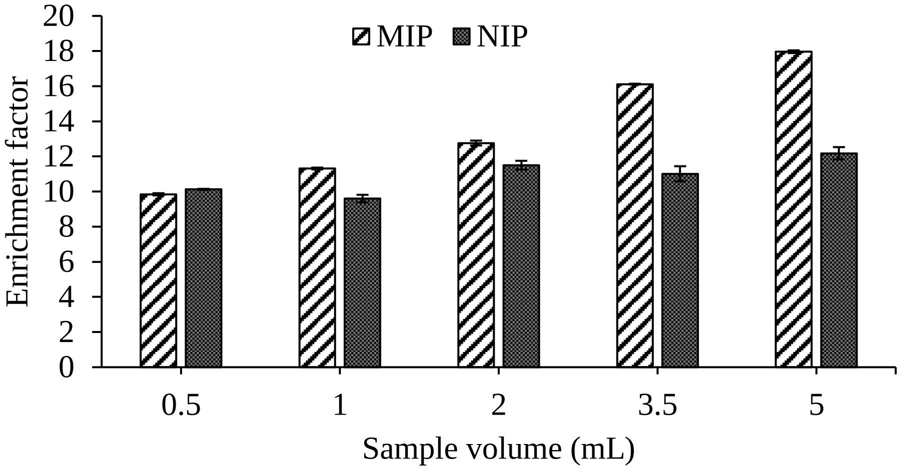

Figure 5.4: Effect of sample mass Effect of sample volume (n = 3, RSD). Experimental conditions mass of glass wool = 30 mg, adsorbent dose = 100 mg, pH = 3, sample concentration = 1.0 mg L^{-1}, eluting volume = 3 mL, eluting solvent = acetonitrile, washing solvent 2:3 v/v (MeOH/H_2O)

5.3.5 Effect of eluting volume

The effect of eluting solvent volume is represented by Figure 5.5. It was performed by increasing eluting solvent volume from 3 to 11 mL. Enrichment factor of pirimicarb by the MIP decreased with increase of eluting solvent volume. This is because of the dilution factor on the analyte retained, (C = n/v). A similar trend was observed with NIP.

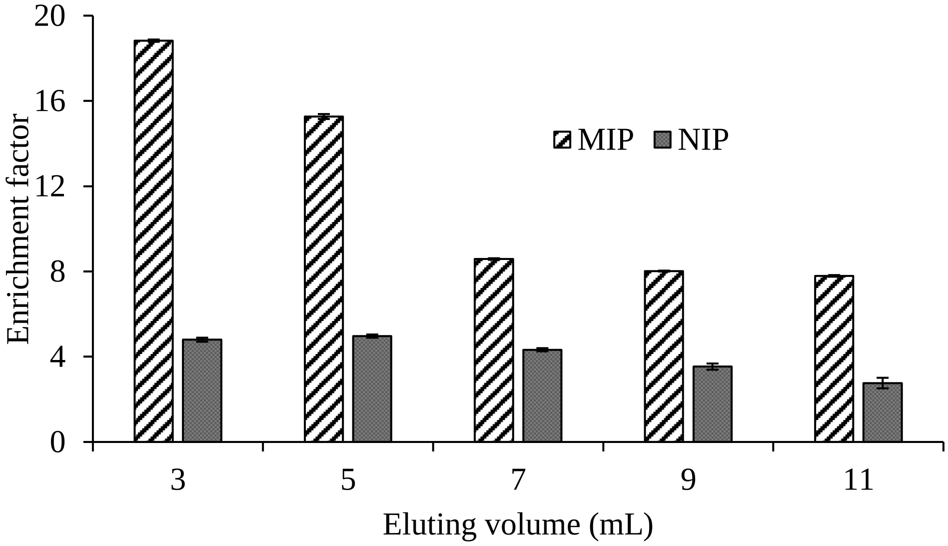

Figure 5.5: Effect of eluting volume (n =3, RSD). Experimental conditions mass of glass wool = 30 mg, sample pH = 3, adsorbent dose = 100 mg, sample concentration = 1.0 mg L^{-1}, sample volume = 5 mL, eluting solvent = acetonitrile, washing solvent 2:3 v/v (MeOH/H_2O)

5.3.6 Effect of concentration

The effect of concentration of the analyte is represented by Figure 5.6. The experiments were performed by changing the concentration of analyte from 0.5 to 2.5 mg L^{-1}. The enrichment factor increased with increase in concentration of the sample. This was because of having more analyte to be retained by the MIP and the NIP. A similar trend was observed by He et al (2015) in extraction of malathion using MIMs and RAM-MIMs where at binding equilibrium *Q* increased with concentration.

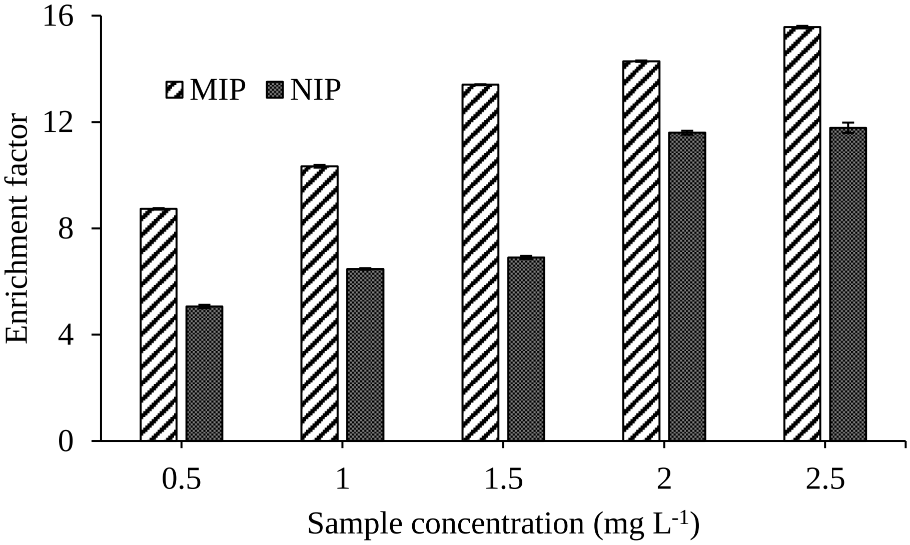

Figure 5.6: 1effectEffect of sample concentration (n = 3, RSD). Experimental conditions mass of glass wool = 30 mg, sample pH = 3, adsorbent mass = 100 mg, sample volume = 5 mL, eluting volume = 3 mL, eluting solvent = acetonitrile, washing solvent = 2:3 v/v (MeOH/H_2O)

5.4 Method validation

The analytical performance of the developed MIP-SPE method was validated through the determination of linearity, sensitivity, repeatability, limits of detection (LOD) and limit of quantification (LOQ) using the optimized MIP-SPE conditions prior to real sample analysis.

The concentration range tested for (propoxur, pirimicarb and carbaryl) was 0.5 and 0.25 mg L^{-1}. Each analyte exhibited good linearity with coefficient of determination (r^2) ranging from Each analyte exhibited good linearity with coefficient of determination (r^2) ranging from 0.959 to 0.999 in Table 1.

Table 5.1: Validation parameters for molecularly imprinted polymer solid phase extraction

Pesticide	Calibration equation	R^2	LOD	LOQ	Linear range (mg L^{-1})
Carbaryl	y = 628719x + 137041	0.9809	0.41	1.24	0.25-2
Propoxur	y = 812825x + 492382	0.959	0.50	1.50	0.5-2.0
Pirimicarb	y = 1148135x + 1454607	0.99	0.62	1.86	0.5-2.5

LOD = limit of detection; LOQ = limit of quantitation; RSD = relative standard deviation

5.5 Applications to orange samples

The analyte recoveries obtained ranged from 59.18 - 84.67%. It was noted that the recovery for pirimicarb was higher compared to the other two analytes. This is expected as pirimicarb was used as template which produces selectivity in terms of shape and functional group. Nevertheless, the other two analytes (propoxur and carbaryl) were also successfully extracted because of the similarity of analogue and chemical structures with the template. The method also showed low limits of detection (n = 3) obtained for all analytes in the range of 0.41 to 0.62 mg L^{-1}. The method indicated repeatability as the RSD ranged from 1.37-9.23. The potential of the developed MIP-SPE method for the selective sample clean-up were investigated in the analysis of oranges. It was found that none of the analytes were detected in the orange samples. This was probably because the carbamates were absent or present at ultra-trace levels in the grape samples.

Table 5.2: Determination of carbamates in orange samples (n=3)

Pesticide	Real sample	Recovery (%) 0.5 mg L^{-1}	RSD	Recovery (%) 0.25 mg L^{-1}	RSD	Recovery (%) 0.1 mg L^{-1}
Carbaryl	<LOD	81.46	4.814	77.545	3.8197	59.18
Pirimicarb	<LOD	84.67	9.226	83.07	6.656	62.50
Propoxur	<LOD	69.427	1.37	A		-

a= not detected

-means not available

5.6 Method comparison of carbamates with various extraction techniques

The efficiency of the presented SPE method for orange samples were compared with other reported methods such as MIP-SPE, SPE-DLLME, SPE-LCMS, SPE-UHPLC AND SPE-LCM in terms of the enrichment factor, linear range, limit of detection, relative standard deviation, extraction time and sample volume. As listed in Table 5.3, the recovery percentage for this method were lower than the other method while limit of detection was small so this method can be used for ultra preconcentration of carbamates from food samples.

Table 5.3: Method comparison for determination of carbamates using solid phase extraction

Method	%Recovery	Linear range	RSD	LOD	LOQ	Sample	Reference
MIP-SPE	99.5-112.1%	2.0-1400 µg kg^{-1}	1.4-7.2	0.6 µg kg^{-1}	2 µg kg^{-1}	Tomato and Pear	Zhou et al., 2010
SPE-DLLME	5.400 -7.650*	0.10- 100 µg kg–1	1.53-8.79	0.005-0.06 µg kg^{-1}	-	Cucumber and Apple	Zhou et al., 2012
SPE-LCMS	81-82	0.0005-0.1 mg L^{-1}	14.2-20.8	.0003-0.003 mg L^{-1}	0.001-0.01 mg L^{-1}	White and red wine	Economou et al., 2009
SPE-UHPLC	95.1-104.7	0.005-5 ng L^{-1}	3.09-0.0097	0.5 ng L^{-1}	1.5 ng L^{-1}	Water	Shi et al., 2014
LCMS	69.427-84.67	0.25-2.5 mg L^{-1}	1.37-9.266	0.412141-0.61961 mg L^{-1}	1.23766-1.860934 mg L^{-1}	Oranges	This method

*Enrichment factor

-means not available

Conclusion

The study demonstrated that MIPs synthesised using pirimicarb were effective for simultaneous extraction of propoxur, carbaryl and pirimicarb. In this method, a sorbent of 100 mg gave optimum results. The method was pH dependent, and the extraction efficiency increased with sample volume. Acetonitrile was used as an eluting solvent with decrease in increase eluting volume. Low LODs of carbamate compounds were achieved, and it was confirmed that the method could be used for the determination of trace level carbamate compounds in real wastewater samples had satisfactory relative recoveries.

Chapter 5

Conclusions and future work

In this section, general conclusions are drawn based experimental findings. future work is also presented in this section.

5.1. Conclusion

Pesticides are helpful especially in farming as they eliminate pests that can destroy crops and they can also be helpful in minimizing diseases such malaria. Misuse of pesticides has led them to be detected in various environmental compartments. Some of the pesticides were banned but they are still detected in the environment due to their long-term persistence. They were distributed into the environment via storage spillage, agricultural runoffs, and leaching. Therefore, it is important to develop methods that can identify them at trace level.

in the first part of this work, we reviewed the challenges faced during template removal from the MIP. The effects of template bleeding were reviewed. Methods of minimising template bleeding which included more washing, use of dummy template and effects that isomerism, monomer-template interaction, and unstable compounds has on the removal on the template removal were reviewed. More washings results in better removal of template but can cause rapture of cavities if overdone so the recommended method that most researchers use is the dummy template which gives good percentage recoveries. Researchers needs to look at computational simulation to select an appropriate solvent as to minimize the use of lots of solvents that does effectively remove the template.

In part 2 carbaryl was used to synthesise dummy template molecularly imprinted polymers. It was synthesized using precipitation technique. The sorbent was used in miniaturised pipette-tip extraction and placed on the tip of the pipette. This technique uses small sorbent and solvents, which is desirable as it minimises the use of lots of hazardous chemicals. The sorbent effectiveness was tested with different optimisation parameters including sample pH, adsorbent dose, eluting solvent, eluting volume, sample volume and loading volume. Significant enrichment factors were obtained. The method was applied in grape sample where 4 carbamates were not detected as they may be present in very low concentration that is beyond limit of detection of the instrument.

In part 3 molecularly imprinted polymers was synthesized using precipitation polymerisation technique with pirimicarb as a template. The MIP was used as a sorbent in Solid phase extraction to preconcentrate pirimicarb. During the method development, different parameters were optimised where good enrichment factors were obtained. the parameters that were investigated included, sample volume, pH, mass, eluting volume, and concentration. The method showed good LOD and RSD which means that the method repeatable and it has potential to analyse carbamates at trace level. It was applied in orange samples where 3 carbamates (pirimicarb, carbaryl and propoxur) were not detected. The carbamates may have not been there on the orange sample or it was present in very low concentration which is not detectable using LCMS.

5.2 Future work

The major drive-in future is to find a method that can simultaneously preconcentrate similar analytes in trace analyses. Multi-template MIPs have been utilised to extract many analytes at once and it has been proven in literature to have better recoveries as they have many active sites. Also, the monomer-template interactions should be taken into consideration when choosing a template in other to enhance enrichment factor.

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Appendix

This section gives supplementary data for all the papers in this dissertation and paper IV which was done with colleagues.

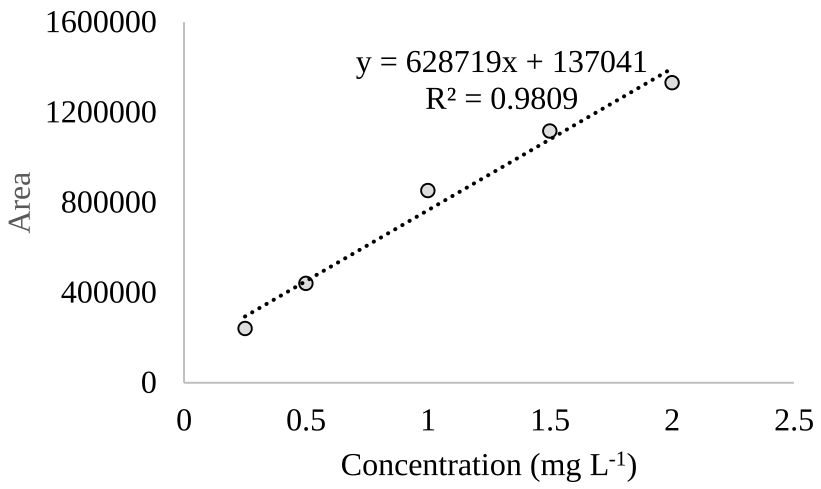


Fig A1: Calibration curve for carbaryl standard for real sample of paper III

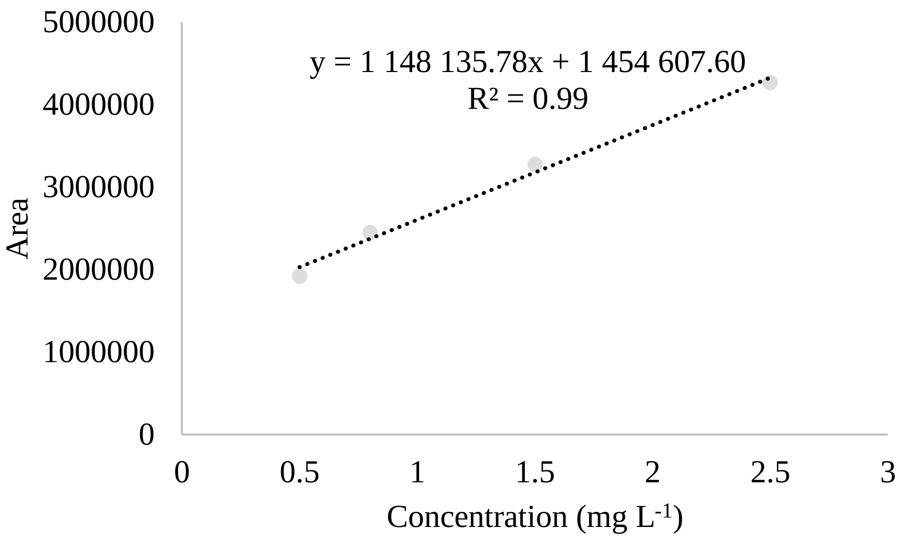


Fig A2: Calibration curve for carbaryl pirimicarb standard for real sample analysis of pirimicarb for paper III

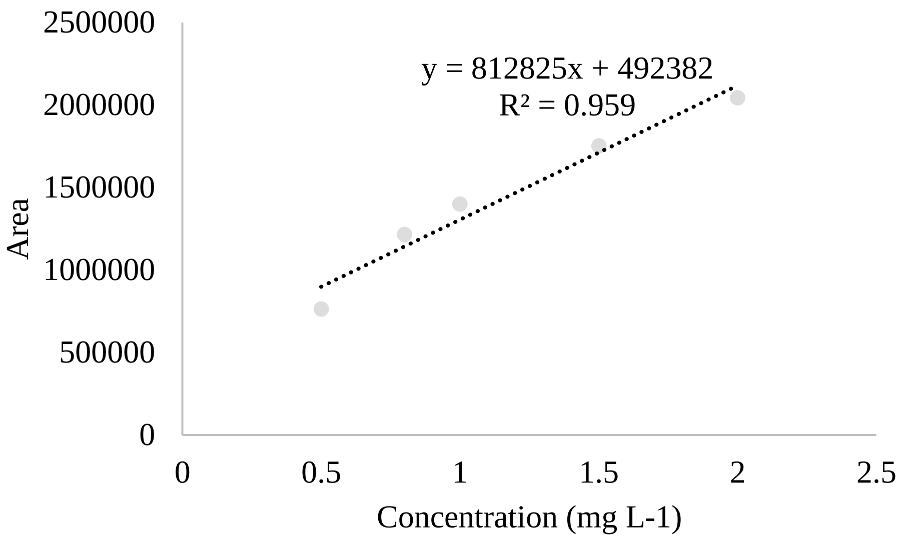
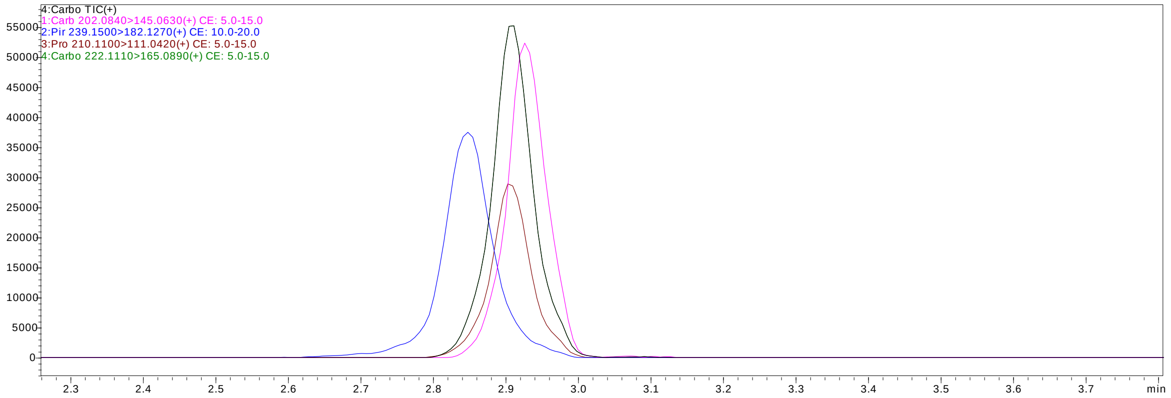


Fig A3: Calibration curve for propoxur standard for real sample analysis for paper III

Applications to grape samples

(1)



(2)

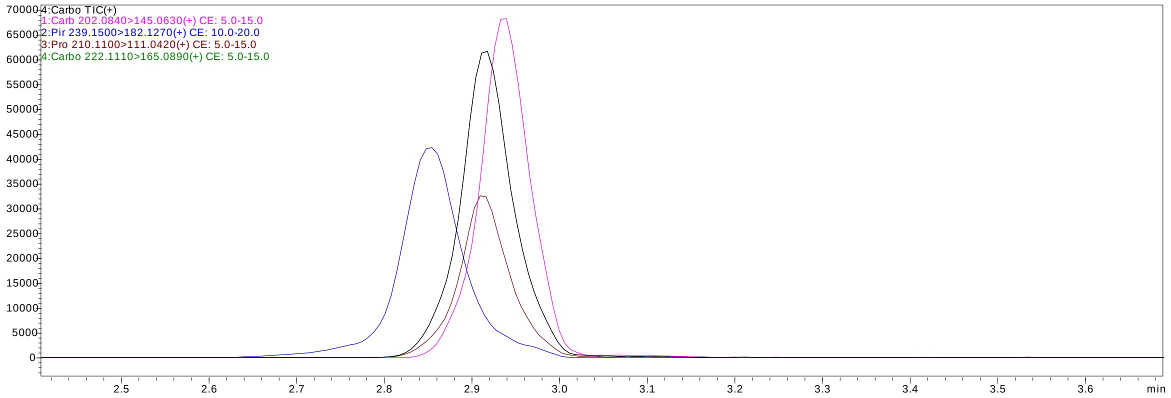


Figure 4A: Chromatograms of grapes samples by MPT-SPE. (1) spiked with carbamates at the concentration 0.5 mg L^{-1} (2) spiked with carbamates at the concentration 0.25 mg L^{-1}

(2)

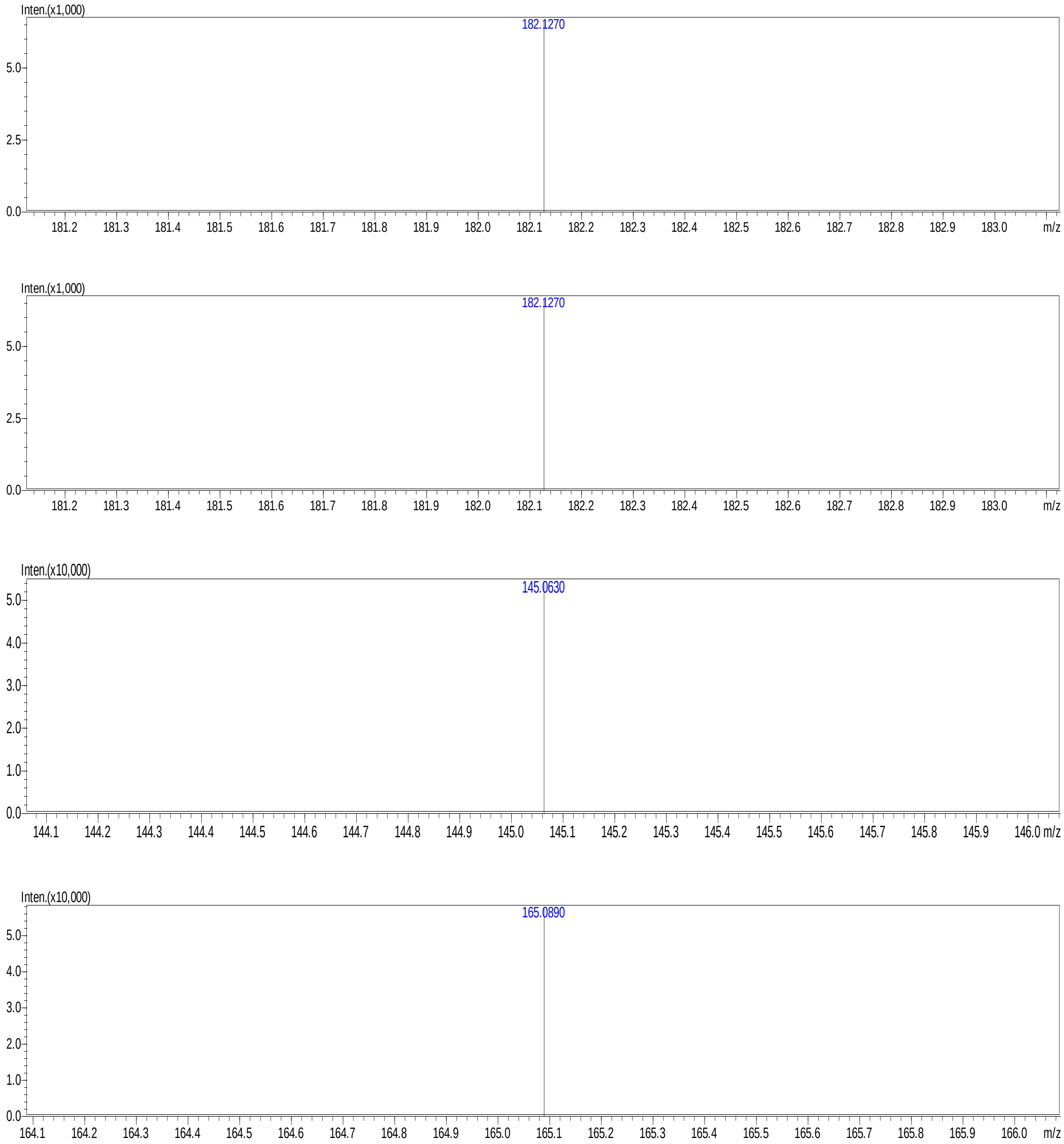


Figure 5A: MS of grapes spiked at 0.5 mg L^{-1} of four carbamates.

Appendix B: Paper IV

Chiral pesticides: Analysis, fate, and toxicity in the environment

Chiral pesticides: Analysis, fate and toxicity in the environment

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ABSTRACT

The use of pesticides for industrial and domestic purposes has increased tremendously in recent years. Herbicides, fungicides, pyrethroids and organophosphates are among the pesticides that are widely used. Most of these pesticides have one or more asymmetric chiral atoms, and therefore, they exhibit stereoisomerism. More often than not, these chiral pesticides are sold as racemates. The enantiomers in the racemates usually have different toxicity to both target and non-target organisms. Sometimes, only one enantiomer is active and the other ones are less or not active but toxic to non-target organisms. Detailed examples to show the enantioselective toxicity of chiral pesticides to both target and non-target organisms are given in this chapter. The need for assessment of the environmental impact of chiral pesticides should not be over-emphasised. Monitoring of chiral pesticides in the environment can be done using chiral separation techniques such as chiral high performance liquid chromatography, chiral gas chromatography, chiral capillary electrophoresis and chiral supercritical fluid chromatography. Thus, this chapter discusses the enantioselective analytical methods that have been developed to enable separation and identification of enantiomers of chiral pesticides. The enantiomers of chiral pesticides can have detrimental effects to the environment even at trace concentrations. Consequently, pre-concentration techniques such as dispersive liquid-liquid micro-extraction, QuEChERS, liquid-liquid extraction and SPE are sometimes employed to enable trace analysis of chiral pesticides and their applications during chiral pesticide analysis are discussed in this chapter.

KEYWORDS

Chiral pesticides, enantiomers, chiral separation techniques, environmental fate, enantioselective toxicity

Introduction

Pesticides have found widespread application in agriculture, industry and homes to control or prevent invasive insects, diseases, and unwanted plant growth (Qu et al. 2016). About 30% of the known registered pesticides are chiral (Caballo, Sicilia, and Rubio 2013; Chen et al. 2016; Cui et al. 2018). Such pesticides include the most frequently used herbicides (Buerge et al. 2019; Caballo, Sicilia, and Rubio 2014), pyrethroid insecticides (Birolli et al. 2019) and organophosphorus insecticides (Chen et al. 2016; Gao et al. 2019). Chiral pesticides are chemicals consisting of one or two enantiomers that have identical physical and chemical properties in achiral environments (Asad et al. 2017; Zhang et al. 2016).

Chirality is defined formally as the geometric property of a rigid object which is not superimposable with its mirror image. It is found throughout biological systems such as basic building blocks of life, such as amino acids, carbohydrates, and lipids as well as in synthetic compounds such as chiral pesticides (Garrison 2011). This chirality is in most cases illustrated with the idea of left and right-handedness. The left hand and right hand are non-superimposable mirror images of each other (Fig. 1A). Two mirror images of a chiral molecule, such as chiral pesticides, are called enantiomers. Just like with hands, enantiomers come in pairs. Chiral isomers have identical structures (same atoms and identical bonds) but they differ in their molecular conformation (+/-, D/L, or R/S). They have undistinguishable physico-chemical properties in achiral environments. When enantiomers are placed in chiral environments, however, they show different behaviours. A chiral environment can either be physical such as plan polarized light or chemical such as a solvent, a reactant or a catalyst (Mohan, Mohan, and Yamsani 2009). The enantiomers of some of the chiral pesticides are shown in Fig. 1B and C. The asymmetric center is usually a carbon bonded to four different substituents in a 3-dimensional arrangement. However, other atoms such as sulfur and phosphorus can also be stereogenic centers (Fig. 2). A mixture of enantiomers is called a racemate or a racemic mixture.

Most chiral pesticides have been commercialised in industry and agriculture as racemic forms (Asad et al. 2017; Maia et al. 2017). Although the enantiomers in the racemic mixture of a chiral pesticide have identical physical and chemical properties, as already alluded to, they usually display different physiochemical and biochemical properties in chiral environments (Qi et al. 2015; Xie, Zhao, Liu, Guo, and Liu 2018). Most of the time, one of the enantiomers in the racemic mixture will have the desired effect on the target species and the other forms will be less active or even inactive (Cheng et al. 2018; Deng et al. 2019). Thus, the inactive enantiomers will be discharged into the environment where it may contaminate the soil and aquatic ecosystems, and may be toxic to non-target species. It is, therefore, imperative that the enantioselectivity of chiral pesticides should be investigated and monitored so as to reduce the burden and toxicity of the inactive enantiomers on the environment (Qi et al. 2015).

The chiral pesticides, once discharged into the environment, may interact with the biotic and abiotic components resulting in their physical and chemical transformations. Some may undergo photo-degradation, become absorbed in the atmosphere through volatilization, become absorbed by living organisms and may even enter into water sources, soil and sediments (Brusseau 2019: Jin et al. 2016). In living organisms, some chiral pesticides may undergo biodegradation, biotransformation and bioaccumulation. The fate of chiral pesticides in the environment depends on their chemical properties, weather, and soil properties (Brusseau 2019) Physical parameters such as solubility and water partition coefficient determine how likely chiral pesticides are to travel through soil, become volatile and how they will readily dissolve in water (Saranjampour et al. 2019). Chiral pesticides may undergo chemical reactions and disappear completely while others may become resistant and persist in the environment where they will cause detrimental effects. Thus, monitoring and analysis of chiral pesticides in the environment is of utmost importance.

Analytical methods for determining enantiomers in contaminated environments include chiral gas chromatography (Xiang et al. 2019; Yao et al. 2019), supercritical fluid chromatography (Jiang et al. 2018; Tao et al. 2018), chiral high performance liquid chromatography (Zhao et al. 2019; Xia et al. 2019) and chiral capillary electrophoresis–ultraviolet detection (Wan Ibrahim et al. 2007). Most of the chiral pesticides, however, are found in the environment in trace amounts and can have detrimental effects on living organisms at these low concentrations. Thus, pre-concentration of the chiral pesticides is sometimes necessary to enable their detection with analytical instruments. A number of techniques have been utilised by researchers for the pre-concentration and enrichment of chiral pesticides. These include the convectional liquid–liquid extraction (de Albuquerque, de Matos, and de Oliveira 2016), solid phase extraction (Gao et al. 2016), magnetic solid phase extraction (Zhao et al. 2019), solid phase micro-extraction (Dallegrave et al. 2016; Gao et al. 2016), sonication assisted extraction (Buerge et al. 2015; Corcellas, Eljarrat, and Barceló 2015) and QuEChERS (He et al. 2018; Tan et al. 2017).

This chapter presents recent techniques used to analyse chiral pesticides in contaminated environments. In addition, the fate and toxicity of chiral pesticides are critically discussed. The use of chiral pesticides is not free of challenges. Thus, the challenges associated with the use and analysis of chiral pesticides are tackled as well in this chapter.

The information in this chapter is going to supplement and provide an update of the already existing reviews and book chapters on chiral pesticides.

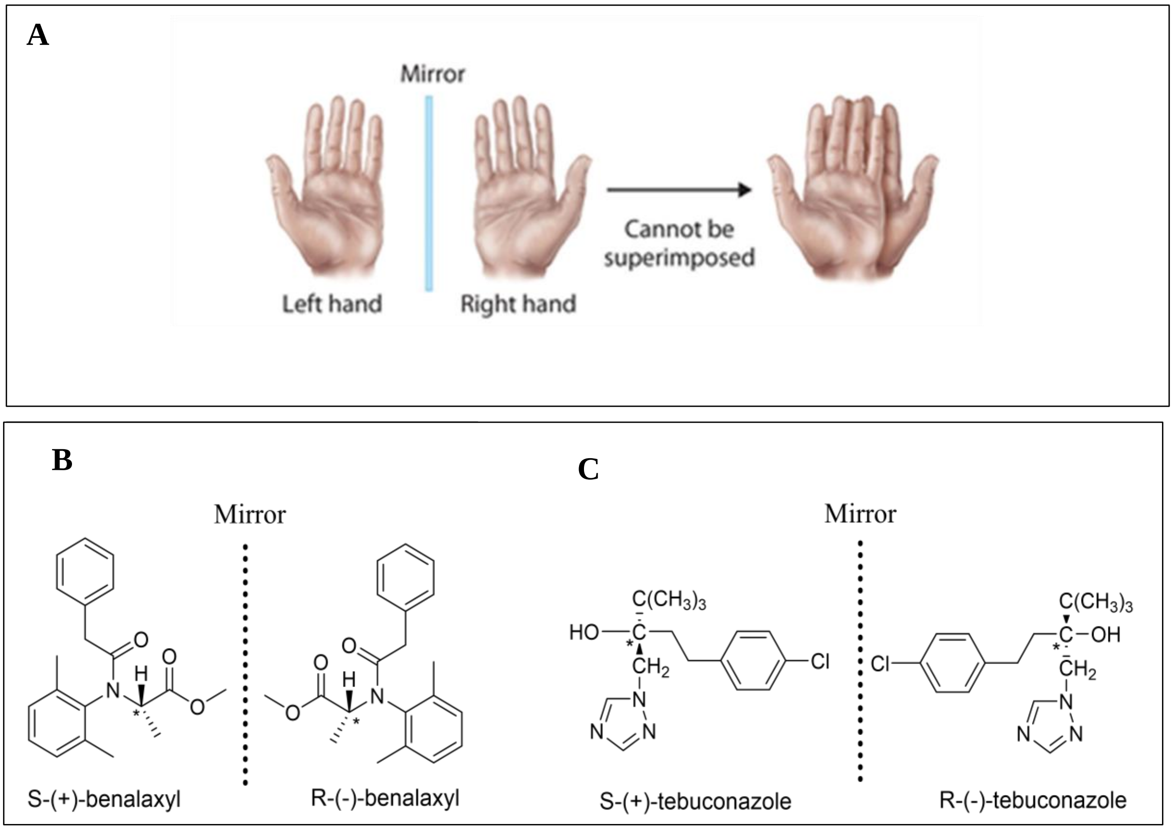


Fig. 1: (a) Illustration of chirality using the right and left-handedness, (b) enantiomers of benalaxyl pesticide and (c) enantiomers of tebuconazole pesticide. **Note:** *- asymmetric centre.

2. Common types of chiral pesticides

2.1. Chiral organophosphates

Chiral organophosphate pesticides are among the most widely used agrochemicals for controlling pests (Gao et al. 2019). Thus, they play a pivotal role in crop protection. Chemically, chiral organophosphates are esters of phosphoric acid or thio-phosphoric acid, with one or more chiral centres (Fig. 2a). The mechanism of their insecticidal activities involves the inhibition of an enzyme called acetylcholinesterase in insects (Zhang et al. 2017). Chiral organophosphates, however, can also be toxic to non-target organisms through the same mechanism. The inhibition occurs through the phosphorylation of the active site of acetylcholinesterase (Shakoori et al. 2017). This enzyme hydrolyses acetylcholine in cholinergic synapses as well as in neuromuscular junctions (Mangas et al. 2016). Thus, its inhibition by chiral organophosphates causes excessive accumulation of acetylcholine in synapses, with the subsequent activation of cholinergic receptors. This results in gastrointestinal distresses, dizziness, headaches, bronchospasm, urination, sweating, lacrimation, muscle weakness, hypertension, liver and kidney damage, coma and ultimately death (Shakoori et al. 2017; Mohammadi et al. 2015).

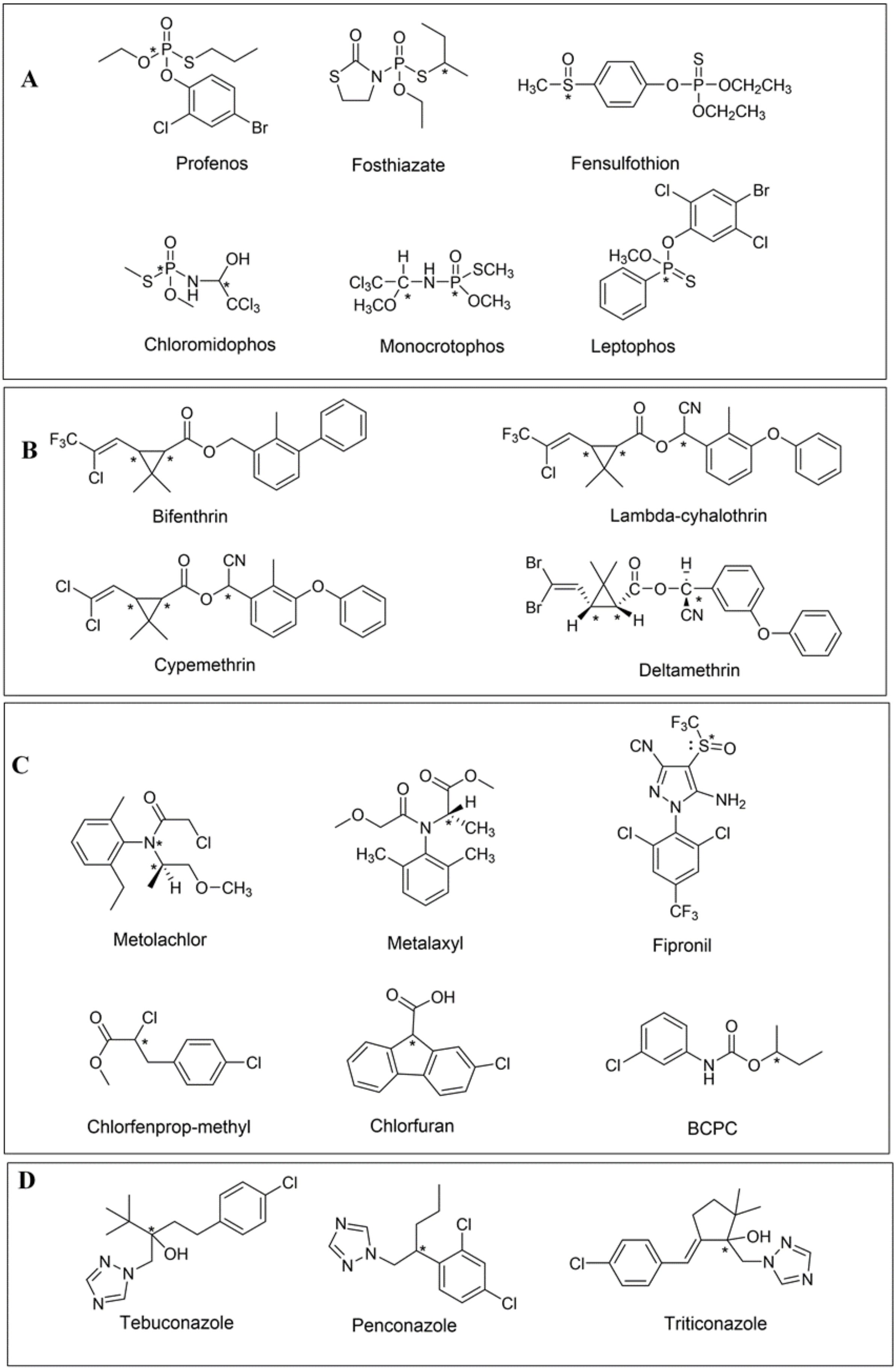

A
Profenos
Fosthiazate
Fensulfothion
Chloromidophos
Monocrotophos
Leptophos
B
Bifenthrin
Lambda-cyhalothrin
Cypemethrin
Deltamethrin
C
Metolachlor
Metalaxyl
Fipronil
Chlorfenprop-methyl
Chlorfuran
BCPC
D
Tebuconazole
Penconazole
Triticonazole

Fig. 2: Structures of some chiral (a) organophosphates, (b) pyrethroids, (c) herbicides and (d) triazole fungicides. **Note:** *- asymmetric centre.

2.2. Chiral pyrethroids

Chiral pyrethroids are synthetic, xenobiotic compounds that have insecticidal properties and are related to the natural insecticides called pyrethrins (Corcellas, Eljarrat, and Barceló 2015). They have widespread use as insecticides in agriculture and forestry as well as for domestic purposes (Zhang et al. 2019; Xiang et al. 2019). Chiral pyrethroids are neurotoxins with one to three chiral centres (Fig. 2b). They are usually sold as racemic mixtures, and their enantiomers usually have stereoselective toxicity to target and non-target organisms (Pérez-Fernández, García, and Marina 2010; Xiang et al. 2019). They affect the sodium channels in the nervous system resulting in the interference with the transmission of nerve impulses. This causes the stimulation of the nervous cells. Consequently, several electric shocks are produced that cause paralysis of the insect (Jiménez-Jiménez et al. 2019). Exposure to chiral pyrethroids causes neurotoxicity, immunity toxicity, development toxicity, and endocrine disrupting toxicity to living organisms (Xiang et al. 2019).

2.3. Chiral herbicides

Chiral herbicides are chemicals manufactured by the agro-industry and they are commonly used to control weeds in farms (Xie, Zhao, Liu, Guo, and Liu 2018; Zhao et al. 2019; Hsiao, Wang, and Yen 2014). Most of the chiral herbicides have one or more pairs of enantiomers and are therefore chiral (Buerge et al. 2015; Asad et al. 2017; Chen, Zhang, et al. 2019) (Fig. 2c). The enantiomers of the chiral herbicides have the same physical and chemical properties in achiral environments but their interaction with enzymes and other chiral biological molecules is different (Díaz Merino et al. 2019). In chiral environments, the asymmetric herbicides show enantioselectivity in their actions (Ye et al. 2015; Caballo, Sicilia, and Rubio 2013).

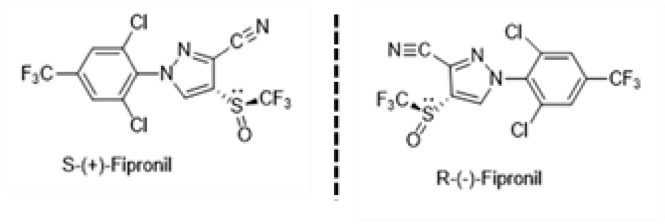
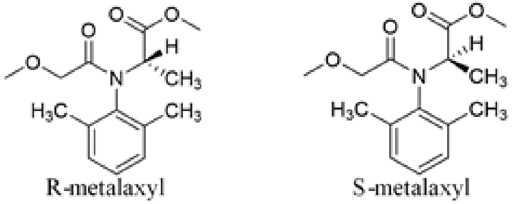
2.4. Chiral triazole fungicides

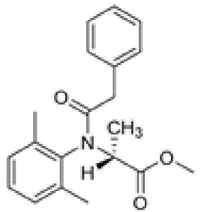
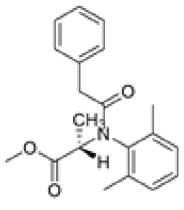
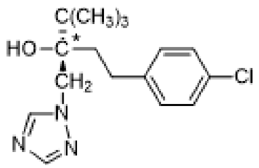
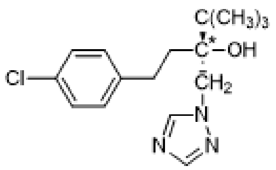
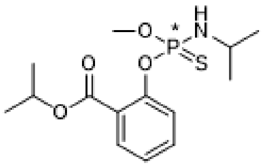
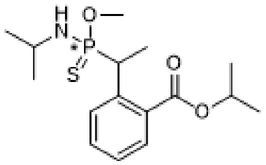
Chiral triazole fungicides are a type of broad-spectrum pesticides that contain the 1,2,4-triazole moiety (Zeng et al. 2019; Wang et al. 2019). Most of them have one or two chiral centres (Zeng et al. 2019) (Fig. 2d). They are widely used in agriculture as racemic mixtures to prevent and treat a wide variety of fungal diseases in crops. The chiral triazole fungicides inhibit ergosterol biosynthesis in the fungi and this is an integral process during the formation of the fungal cell wall (Zhang et al. 2018; Cui et al. 2018; Tong et al. 2019). Thus, this forms the basis of their anti-fungal activities on fruit, vegetable, legume, and grain crops. In addition, racemates of triazole fungicides are also extensively used in antifouling agents, textiles, leather, wood preservatives, adhesives, and paints (Buerge et al. 2016; Jiang et al. 2018). The chiral triazole fungicides are moderately lipophilic and highly stable since they have long chemical and photochemical half-lives (Zhang et al. 2018; Youness et al. 2018). Thus, they can be easily transported to different environmental media where they accumulate and cause detrimental effects to fauna and flora. In addition, the chiral triazole fungicides have low biodegradability, and this makes them persistent in the environment (Wang et al. 2019).

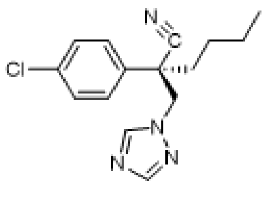
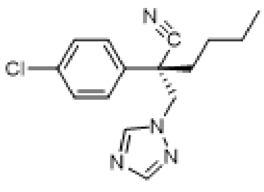
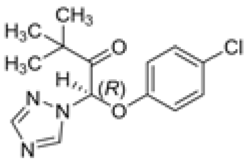
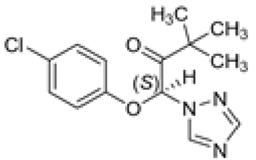
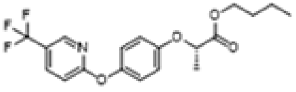
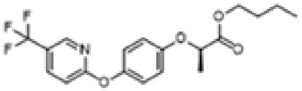
3. Physico-chemical properties of chiral pesticides

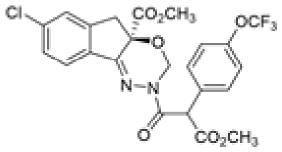
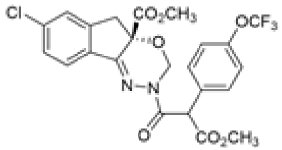
The physico-chemical properties of some of the chiral pesticides are shown in Table 1. In achiral environments, enantiomers have the same physico-chemical properties such as water solubility, vapor pressure and dissociation constant. The behaviour of chiral pesticide residue in the environment is related to physicochemical properties (Lozowicka et al. 2015). Physico-chemical properties determine the environmental fate of chiral pesticides such as degradation and overall pollution potential. N-octanol/water partition coefficient (K_{ow}) is used to estimate the transport and the exposure of chiral pesticides through a different compartment, bio-concentration factor and acute toxicity in aquatic organisms (Saranjampour et al. 2019). Solubility parameter is important for migration potential and persistence in the environment and chiral pesticides having large solubility parameter tend to transfer into water and subject to water flow (O A. Environmental and Pollution Science). For example, imazethapyr, which is a chiral herbicides pesticide, was found in the groundwater of Tibagi River, Brazil due to it having relatively high-water solubility and moderate persistent in soil (Shen et al. 2019). Pyrethroids, on the other hand, have low water solubility and high octanol/water partition coefficient. Consequently, pyrethroids can partition into the organic compartment of sediment and can travel to surface water and sediments (Ulrich et al. 2017). Even though enantiomers have the same physico-chemical properties in achiral environment, in chiral environments, they are enantioselective and result in having different toxicity as well as degrading differently as summarized in Table 1.

Table 1 Physio-chemical properties of different chiral pesticides.

Chiral pesticide	Structure	Water solubility (mg L^{-1})	Behaviour of enantiomers in different environments	K_{ow}	pka values
Fipronil		1.9-2.4	S-(+)- enantiomer shows higher level of genomic DNA methylation than R-(-)- fipronil in zebrafish embryos	4.0	-
Metalaxyl		8.40	S-metaxyl degrade faster at pH < 4 in anaerobic and aerobic soil than R-metaxyl (Celis et al. 2015)	1.65	Much less than zero

Benalaxyl	 S-(+)-benalaxyl  R-(-)-Benalaxyl	28.6	There is a significant conversion of the R-enantiomer of benalaxyl to the S-form in the plasma of mice	3.47×10^{3}	No dissociation
Tebuconazole	 (+)-*S*-tebuconazole  (-)-*R*-tebuconazole	36	R-tebuconazole was found to have greater toxicity to non-target organisms as well as greater fungal activities	3.7	2.3
Isophenphos-methyl	 (S)-(+)-isofenphos-methyl  (R)-(-)-isofenphos-methyl	22.1	R-isophenphos-methyl degrade faster than its S-form in river water	4.12	-

Myclobutanil	 R-enantiomer	 S-enantiomer	142	R-(-)-myclobutanil may stay longer in the body and cause harm while S-(+)-myclobutanil may not cause any harm as they are eliminated by the liver	2.94	-
Triadimefon	 R-(-)-triadimefon	 S-(+)-triadimefon	71.5	RR-(+)-triadimefon produced by R-(-)-triadimefon gives highest fungal activities while RR-(+)-triadimefon produced by R-(-)-triadimefon mostly bioaccumulate in lizard (Shen et al. 2017)	2.77	-
Fluazifop-butyl	 R-fluazifop-butyl	 S-fluazifop-butyl	1.00	R form of fluazifop-butyl degraded faster than its R form in Beijing soil and Anhui soil (Qi et al. 2016)	4.5	-

Indoxacarb	 (-)-(R)-indoxacarb  (+)-(S)-indoxacarb	0.2	(+)-S-indoxacarb Dissipated fast in all three tea farms than (-)-R-indoxacarb (Zhang et al. 2015)	4.65	-

Note: - means no data

4. Environmental fate of chiral pesticides

4.1. Enantioselective biotransformation of chiral pesticides

Chiral pesticides can undergo chemical modifications due to the various metabolic processes that occur in living organisms (Buerge et al. 2016; de Albuquerque et al. 2018; Asad et al. 2017; Wang et al. 2020; Zhang et al. 2019). This phenomenon is called biotransformation, and it usually results in the formation of various metabolites in organisms that may have greater persistency and toxicity in the environment (Gao et al. 2019). Preferential metabolism of the enantiomers of chiral pesticides have been observed in living organisms by many researchers (Zhan et al. 2018; Xie et al. 2019; Shen et al. 2017). Thus, stereo-selective metabolism of chiral pesticide enantiomers in different living organisms is a common occurrence (Wang et al. 2020).

Enantioselective biotransformation of chiral fungicides is common in animals (Zhang et al. 2019; Xie et al. 2019). For instance, Shen et al. (2017) studied metabolism of triadimefon and its chiral metabolite triadimenol in lizards. Their results indicated that triadimefon could undergo metabolic transformation in lizards resulting in the formation of triadimenol. The biotransformation of triadimefon to triadimenol in lizards involved the reduction of its carbonyl group to a hydroxyl group. Enantioselectivity was observed in the results and there was preferential metabolism of the S-enantiomer of triadimefon. In the same vein, Wang et al. (2020) studied the metabolism of tridiamenol in *Eremias argus* lizards. Tissue distribution studies revealed the occurrence of stereoselective metabolism of tridimenol. Their results showed that triadimenol had a greater fungicidal activity than triadimefon as well as greater toxicity on the environment. On the other hand, Xie et al. (2019) investigated the biotransformation of prothiocanazole fungicide in Chinese lizards. Their results showed that prothioconazol was bio-transformed to a chiral metabolite called prothioconazole-desthio in Chinese lizards. Prothioconazole-desthio was found to be more persistent and toxic to living organisms as compared to prothioconazole. In another study, Gao et al. (2019) investigated the biotransformation of ethiprole in algae. Fig. 4 shows their proposed metabolic pathway of ethiprole in algae and water. Their results showed enantioselective biotransformation as R-ethiprole was preferentially metabolised in algae as compared to S-ethiprole. The results also indicated that most of the metabolites were more toxic than ethiprole.

Zhan et al. (2018) studied the biotransformation of an organochlorine pesticide called acetofenate in rabbits. The results revealed that acetofenate was rapidly bio-transformed into its chiral metabolite, acetofenate-alcohol, in rabbits. Enantioselective biotransformation was observed since there was preferential metabolism of (-)-acetofenate to (-)-acetofenate-alcohol.

Wang et al. (2017), on the other hand, investigated enantioselective biotransformation of benalaxyl fungicide in mice. Biotransformation of benalaxyl in tissues and plasma of mice exhibited enantioselectivity with the enrichment of the (-)-S-enantiomer. In another study, Gao et al. (2020) studied the metabolic transformation of flufiprole pesticide in *Chlorella pyrenoidosa*. The enantiomers of flufiprole were mainly transformed to flufiprole amide and detrifluoromethylsulfinyl flufiprole in culture medium but various metabolites, which included amide derivatives and fipronil, were formed in *Chlorella pyrenoidosa*. The toxicity of fipronil, from the results obtained, was the highest and this metabolite poses serious problems to the environment.

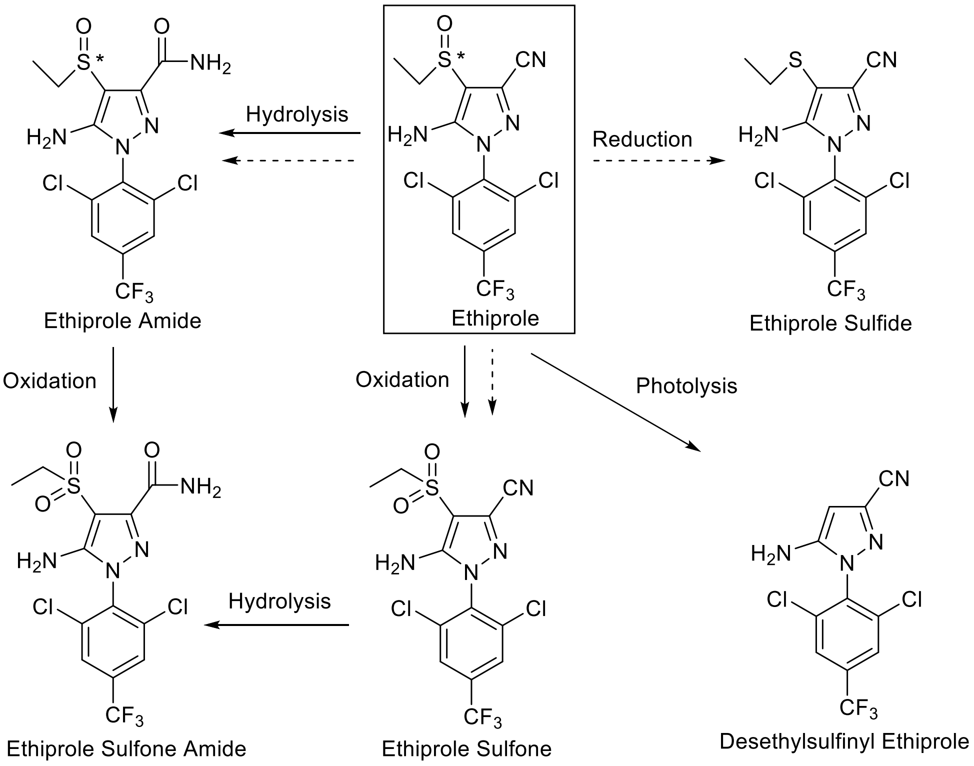


Fig. 4: Metabolic pathway of ethiprole (solid arrow represents transformation in water and dotted arrow represents transformation in algae) (Gao 2019).

4.2. Enantioselective biodegradation of chiral pesticides

Biodegradation is among the common environmental fates of most chiral pesticides (Wang et al. 2015; Maia et al. 2017). It is an effective natural method to eliminate residues of asymmetric pesticides from contaminated environments. It involves the breakdown of the chiral pesticide residues into forms that are compatible with the environment (Talab et al. 2018;

Gao et al. 2020). Enantioselectivity is a common occurrence during biodegradation of most of the enantiomers of chiral pesticides (Birolli et al. 2019; Nillos et al. 2009; Wang et al. 2015).

Cyhalothrin is a chiral pyrethroid that consists of four enantiomers, which are (lR,3R,αR) (lS,3S,αS), (1R,3R,αS) and (lS,3S,αR). The commercial formulation of cyhalothrin is called (±)-lambda-cyhalothrin. The enantiomer of cyhalothrin that has the highest insecticidal toxicity is the (1R,3R,αS)-isomer. Birolli et al. (2019) investigated the biodegradation of (±)-lambda-cyhalothrin with a consortium of bacteria. The biodegradation pathway they proposed is shown in Fig. 5a. Their results showed that the (1R,3R,αS)-enantiomer, the most toxic one, was preferentially biodegraded by bacteria. This preferential biodegradation of (1R,3R,αS)-enantiomer in the environment reduces the risk of (±)-lambda-cyhalothrin to non-target organisms.

Chiral pesticides make their way into the soil through water leaching, field application and air deposition (Liu et al. 2016). Soil and sediments are the major compartments where chiral pesticides are usually deposited and they are more likely to settle in these compartments affecting the overall dispersion of pesticides to the environment (Vryzas et al. 2018). Due to their interaction with matter, some chiral pesticides end up in contact with microorganisms in the soil resulting in their biodegradation. During biodegradation of chiral pesticides, there is preferential interaction between different enzymes from the microorganisms with each enantiomer (Asad et al. 2017). Thus, biodegradation of chiral pesticides in the soil exhibit stereoselectivity. For instance, the enantiomers metalaxyl degrade differently in soil with the S-enantiomer degrading rapidly at pH < 4 in anaerobic and aerobic soil as compared to the R-enantiomer. On the other hand, the R-enantiomer degrade faster than the S-form at pH > 5 in aerobic soils (Celis et al. 2015). In the same vein, Zhang et al. (2018) studied the biodegradation of the enantiomers of ethiprole pesticide in soil. The results also showed enantioselective degradation of this chiral pesticide.

Chiral pesticides may also reach various water bodies through different mechanisms when applied in agriculture. They often bypass wastewater treatment and end up into natural aquatic systems. Reuse of wastewater saves water, but the nature of wastewater can have an impact on the discharge of pesticides to the environment. When wastewater is discharged into the environment, chiral pesticides may find their way into the environment resulting in enantioselectivity by chiral pesticides in the environment. For an example, antifungal was investigated whereby it was found that in untreated wastewater the amidazole were racemic to weakly non-racemic while during the treatment they showed weak enantioselectivity. Also, their enantiomeric composition and fractions of dissolved and suspended antifungals was different suggesting a different behaviour (Huang et al. 2013). The by-product of wastewater is sewage which when applied to the environment might influence the enantioselectivity and persistence of benaxyl and can also increase the degradation of benalaxyl (Jing et al. 2017).

Some pesticides show little change in concentration of influents and effluents as they do not degrade via conventional wastewater treatment. For example, the influents and effluents concentration of mecoprop in wastewater were similar they were ranging between 0.03 and 2 mg L^{-1} which suggested that mecoprop was not degraded during wastewater treatment (Escolá Casas et al. 2017). Also, the enantiomeric fraction (EF) which quantify stereoisomeric composition indicates enantioselectivity when it changes from influents and effluent. The EF values of meconazole in Table 2 indicates slight enantioselectivity as the EF value on the influent was 0.497-0.505 and 0.468 in the effluent (Hung et al. 2012). The influent and effluent show a small change suggesting that the biological action in the sewage system did not change the enantiomeric composition of the pesticide (Zhao et al. 2018).

The biodegradation of pesticides in wastewater depends on factors such as pH, dissolved organic matter and electrolyte composition (Masbou et al. 2018). Some researchers have studied the biodegradation of chiral pesticides in water. Youness et al. (2018) investigated the biodegradation of the fungicide tebuconazole by *Baccillus sp*. bacteria in aqueous solutions. Their proposed biodegradation pathway of tebuconazole is shown in Fig. 5b. Their results exhibited stereo-selectivity as there was preferential degradation of the S-enantiomers as compared to the R-enantiomer. This implied that there would be elevated concentrations of the R-enantiomer in the environment. This would pose serious environmental issues since the R-enantiomer of tebuconazole was found to have greater toxicity to non-target organisms as well as greater fungicidal activity.

Table 2: Concentration of different chiral pesticides in influents and effluents of different wastewater treatment plants

Name of chiral pesticide(s)	Location of the WWTP	Influent (ng L^{-1})	Effluent (ng L^{-1})	Removal efficiency (%)	EF values for influent	EF values of effluent	Reference
Enantiomers of epoxiconazole, hexaconazole, myclobutanil, metalaxyl and napropamide	Sheyang	11.9 - 48.9	3.5 - 18.2	62 - 77	0.49 - 0.51*	No significant alteraction	Zhao et al. 2018
Fipronil	North Carolina	131 ± 30	119 ± 70	-	-	-	Mcmahen et al. 2016
Fipronil	Southwestern U.S.	12 - 31	28 ± 6	44 ± 4	-	-	Supowital 2015
Banaxyl	Beijing, China	-	-	-	-	The EF value of S-benalaxyl was always Less than 0.5 indicating	Jing et al. 2017

						great abundance of S-enantiomer residues	
Ketonazole	Guangzhou	7 - 45	-	-	0.484 - 0.485	0.477	Huang et al. 2013
Miconazole	Guangzhou	0.5 - 9.0	-	-	0.497 - 0.527	0.468	Huang et al. 2013
Miconazole	Guangzhou	3.7 - 6.3	6.6 - 7.0	-	0.504 - 0.497	0.468	Hung et al. 2012
Ketonazole	Guangzhou	42.7 - 45.5	0.54 - 0.61	-	0.484	0.485	Hung et al. 2012

Note: *The Ef observed was of four pesticides, metalaxyl was not included. The four pesticides are still produced as racemates; - means no data.

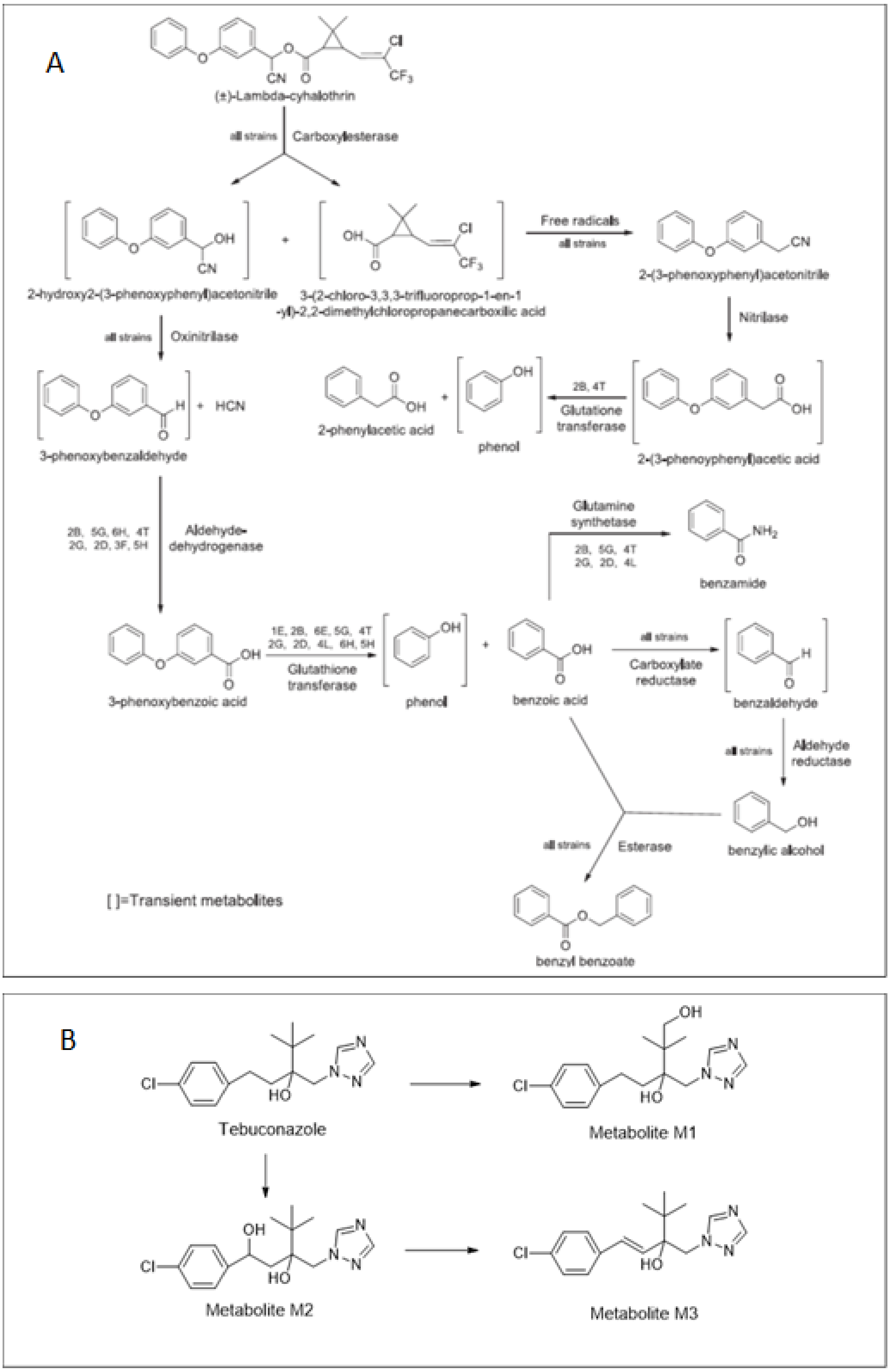


Fig. 5: Biodegradation pathway for (a) lambda-cyhalothrin (Birolli et al. 2019) (b) tebuconazole (Youness et al. 2018)

4.3. Enantioselective bioaccumulation of chiral pesticides

Bioaccumulation is the gradual increase of a chemical's concentration in an organism, and some chiral pesticides exhibit this phenomenon (Cui et al. 2018; Gao et al. 2014; Wang et al. 2018; Yin et al. 2017). During bioaccumulation, the rate of absorption of the chiral pesticides would be faster than its removal rate in the organism through processes such as catabolism and excretion. Chiral pesticides that are lipophilic and have longer half-lives have exhibited greater tendency of bioaccumulation (Cui et al. 2018; Zhou et al. 2018). Thus, chiral pesticides continue to accumulate in the food chain until they reach toxic levels, surpassing their maximum allowable limits (MALs). The concentrations of chiral pesticides are strictly controlled by many regulatory bodies in the world that set the MALs for toxic substances in the environment

The potential negative long-term health impacts of chiral pesticides to organisms' humans is mostly unknown which led to regulatory organisations stipulating different amount of residue that a person should be allowed to take. The presence of pesticides in different compartment is regulated by national and international rules that establish the MAL concentration of pesticides (Table 3). These bodies include WHO, FAO, EU and USEPA. European Union's stipulates a limit of 0.1 ug/L for individual pesticides in water that are allowed for human consumption. Chiral pesticides are assessed as racemates even though they have different toxicity in the environment. For example, R enantiomer of metalaxyl is sold as a single enantiomer but it is regulated by EFSA as combination of R and S in the daily intake in a human body (O A 2016). Low concentrations of chiral pesticides are considered safe for human consumption in food and water (Table 3). Despite all these efforts to minimize exposure of humans to high amounts of chiral pesticides in food and water, their bioaccumulation in the food chains may cause increase in their concentrations beyond MAL values.

Enantioselectivity have been observed during bioaccumulation of chiral pesticides in living organisms (Cui et al. 2018; Yin et al. 2017; Zhou et al. 2018). Many researchers have extensively studied the process of enantioselective bioaccumulation of chiral pesticides (Cui et al. 2018; Yin et al. 2017; Wang, Li, and You 2019; Zhou et al. 2018). For instance, Wang et al. (2019) studied the bioaccumulation of triticonazole and prothioconazole in earthworms. The results showed that the bioaccumulation of triticonazole and prothioconazole in earthworms was enantioselective. The *R*-triticonazole and *S*-prothioconazole exhibited preferential bioaccumulation in the earthworms. In a similar study, Cui et al. (2018) investigated the bioaccumulation of tebuconazole in earthworms. Tebuconazole is a chiral fungicide that is a sterol demethylation inhibitor and is widely used to control pests in crops such as cereals, fruits and vegetables. Cui et al. (2018), during their bioaccumulation study, exposed earthworms to rac-tebuconazole-contaminated soil and analysed the concentrations of the enantiomers periodically. Their results showed that the enantiomers of tebuconazole were enantioselectively bio-accumulated in earthworms. The S-(+) enantiomer had priority as compared to the R-form during bioaccumulation of tebuconazole in earthworms. On the other hand, Yin et al. (2017) studied the bioaccumulation of furalaxyl pesticide in *Tenebrio molitor* larvae. The larvae were exposed to rac-furalaxyl and the concentration of the enantiomers were measured regularly using LC-MS/MS. It was observed that the bioaccumulation capacity of the two enantiomers of furalaxyl were generally very low. The results, however, showed a preferential bioaccumulation of S-furalaxyl as compared to R-furalaxyl. Thus, the bioaccumulation of furalaxyl also showed enantioselectivity.

Table 3: Maximum allowable limits of different groups of chiral pesticides by several international standards regulating bodies in different environmental compartment.

Regulatory Organisation	Pesticide(s)	MAL	Environment compartment	Reference
EFSA	Benalaxyl	0.60 mg kg $^{-1}$	Table grapes	O A 2013
		0.10 mg kg $^{-1}$	Wine grapes	
		0.02 mg kg $^{-1}$	Potatoes	
Codex	Metalaxyl	0.50 mg kg $^{-1}$ in 7 days	Tomato	Malhat 2017
DOH	Myclobutanil	0.05-0.5 mg kg $^{-1}$	Cucurbits, dry beans and pears	O A 2020
Codex	Pebnconazole	0.1 mg kg $^{-1}$	peach, plum, plum apricot and mango	Abd-Alrahman et al. 2012

EU	Indoxacarb	0.1 mg kg $^{-1}$	brown rice	Shi et al. 2018
EU and Japan	Fipronil	5 and 2 µg kg^{-1}	Food and agricultural products	Chen et al. 2018
US	Metalaxyl and its metabolite	6.0 mg L^{-1} 20.0 mg L^{-1} 0.5 mg L^{-1}	Forge Hay Almond	Pohanish 2015
EFSA	Metalaxyl and metalaxyl-M	daily intake of 0.08 mg/kg body weight per day and an acute reference dose of 0.5 mg kg $^{-1}$ bw	Human body	O A 2016
DOH	Penconazole	0.02-0.2 ng L^{-1}	Apples, cucurbits, grapes, pears and peas	O A 2020

Note: WHO = World Health Organisation, EFSA = European Food Safety, DOH = Department of Health, EU = European Union

4.4. Enantiomerisation

The enantiomers of chiral pesticides are not equally active towards the target organisms. In most cases, as already alluded to, one enantiomer has more activity and toxicity towards the target pest as compared to others. Thus, environmental risk of chiral pesticides can be reduced by using them in their enantiopure forms (Shi et al. 2018; Xie et al. 2018). This involves the manufacture of enantiopure formulations of the chiral pesticides so that the active enantiomer is the one applied to crops. In some cases, however, some asymmetric pesticides may be configurationally unstable under certain conditions and may undergo stereochemical inversion (Talab et al. 2018; Yin et al. 2017). This phenomenon is called enantiomerization and is one of the fates of many chiral pesticides in the environment.

Many researchers have investigated the enantiomerization of chiral pesticides under different environmental conditions (Gao et al. 2014; Wang et al. 2017; Yin et al. 2017; Talab et al. 2018). For instance, Talab et al. (2018) investigated the effect of soil pH on the enantiomerization of tridimefon pesticides using alkaline soil from Beijing, acidic soil from Wuhan and neutral soil from Changchun, China. The results revealed significant interconversion of the enantiomers of tridimefon at diffirent pH conditions. The alkaline soil from Beijing exhibited faster interconversion of the enantiomers of tridimefon than neutral soil from Changchun, while acidic soil from Wuhan showed no interconversion. In a similar study, Yin et al. (2017) investigated enantiomerization of furalaxyl pesticide in *Tenebrio molitor* larvae. Furalaxyl is a chiral fungicide that is manufactured in the agro-industry as a racemic mixture. Yin et al. (2017) results revealed that furalaxyl undergo significant enantiomerization in *Tenebrio molitor* larvae under S- or R-exposure. In the same vein, Guo et al. (2014) studied the enantiomerization of a chiral fungicide called matalaxyl in *Tenebrio molitor* larvae. The larvae were exposed, in turn, to enantiopure R-metalaxyl and S-metalaxyl. It was observed that there was interconversion between the R- and S-enantiomers. Guo et al. (2014) attributed the enantiomerization to the presence of an enzyme in *Tenebrio molitor* larvae that catalysed the interconversion.

Enantiomerization may have negative effects to the environment. This usually occurs when the active enantiomer is converted to the inactive form that has greater toxicity and persistency in the environment. For instance, Wang et al. (2017) studied the enantiomerization of benalaxyl in mice. Benalaxyl is a systemic chiral fungicide that is widely used on crops such as soyabeans, grapes, tomatoes, potatoes and onions to control diseases caused by *Oomycetes*. It has one asymmetric centre and it, therefore, consists of two enantiomers. The R-enantiomers has been reported as the one with fungicidal activity. The results of Wang et al. (2017) revealed that there was a significant conversion of the R-enantiomer of benalaxyl to the S-form in the plasma of mice. Thus, enantiomerization, in this case, favoured the formation of the inactive S-enantiomer. Consequently, this caused the excretion of large quantities of the S-enantiomer into the environment, putting non-target organisms at risk.

5. Enantioselective action of chiral pesticides on target organisms

Stereo-selectivity of chiral pesticides has received particular attention from researchers in recent years (Duan et al. 2018; Zhao et al. 2019; Tong et al. 2019; Li et al. 2018). This was necessitated by the realisation that the enantiomers of chiral pesticides exhibited significant differences in their bioactivity on target organisms (Cui et al. 2018; Qi et al. 2015). Thus, a lot of research efforts were directed towards the establishment of the enantioelective activity of chiral pesticides on target organisms. Many researchers have found out that the enantiomers of chiral pesticides do not have same activity on the target organisms (Zhang et al. 2019; Tong et al. 2019; Xie, Zhao, Liu, Guo, and Liu 2018; Hsiao, Wang, and Yen 2014) (Table 4). One enantiomer is usually more active than the others on the target organism.

Many researchers have studied the bioactivity of chiral fungicides in recent years (Cui et al. 2018; Kaziem et al. 2020; Li et al. 2018; Zhang et al. 2019) (Table 4). Chiral fungicide are chemical compounds that are used to kill parasitic fungi as well as their spores. They cause inhibition of the growth of the fungal cells. Research has shown that the bioactivity of chiral fungicides is enantioselective. For instance, Zhang et al. (2019) investigated the bioactivity of prothioconazole on fungi and they found that its action was stereo-selective. The enantiomers had different bioactivities. R-prothioconazole was more potent on fungi as compared to S-prothioconazole. Tong et al. (2019) studied the bioactivity of tetraconazole on *Rhizoctonia cerealis* and *Fusarium graminearum.* Their results also established the enantioselective nature of the bioactivity of tetraconazole. The R-enantiomer had greater fungicidal activity than the S-enantiomer. In a similar study, Kaziem et al. (2020) investigated the activity of epoxiconazole on *Chlorella vulgaris*. Their results showed that S,R-epoxiconazole caused greater inhibition of the growth of *Chlorella vulgaris* as compared to R,S-epoxiconazole. Thus, chiral fungicides exhibit enantioselective bioactivity on their target organisms.

Some researchers studied the bioactivity of chiral herbicides (Hsiao, Wang, and Yen 2014; Qi et al. 2015; Xie, Zhao, Liu, Guo, and Liu 2018; Zhao et al. 2019) (Table 4). Chiral herbicides are chemical compounds that are used to kill undesirable plants or weeds. Research work has established stereoselectivity in the bio-action of most of the chiral herbicides. For instance, Zhao et al. (2019) studied the bioactivity of the enantiomers of metolachlor on *Echinochloa crusgali*. Metolachlor retard growth of *Echinochloa crusgali* through inhibition of gibberellic acid biosynthesis. Their results showed that S-metolachlor had greater herbicidal activity on *Echinochloa crusgali* than R-metolachlor. In a similar study, Xie et al. (2018) investigated the activity of lactofen also on *Echinochloa crusgali* weed and enantioselective bioactivity was observed. They found out that R-lactofen was more potent on *Echinochloa crusgali* than S-lactofen. Thus, enantioselective bioactivity is a general occurrence when chiral herbicides are used in the agro-industry.

Table 4: Enantioselective bioactivity of the enantiomers of chiral pesticides on target organisms

Class of pesticide	Chiral pesticide studied	Target organism studied	Bioactivity on target organism	Active enantiomer	Reference
Triazole fungicide	Prothioconazole	Various types of fungi	Inhibition of biosynthesis of ergosterol and deoxynivalenol	R-prothioconazole is more active than S-prothioconazole	Zhang et al. 2019
Triazole fungicide	Tetraconazole	*Rhizoctonia cerealis* and *Fusarium graminearum*	Inhibition of biosynthesis of ergosterol	R-(+)-tetraconazole had greater fungicidal activity than S-(-)-tetraconazole	Tong et al. 2019
Herbicide	Metolachlor	*Echinochloa crusgali*	Inhibition of gibberellic acid biosynthesis	S-metolachlor more active than the R-isomer	Zhao et al. 2019
Triazole fungicide	Tebuconazole	*Botrytis cinerea*	Inhibition of growth of *Botrytis cinerea*	R-tebuconazole more active than S-tebuconazole	Cui et al. 2018

Herbicide	Beflubutamid	Dicotyledonous weeds	Inhibition of carotenoid biosynthesis	(-)-beflubutamid more active than (+)-beflubutamid	Buerge et al. 2013
Triazole fungicide	Myclobutanil	*Fusarium verticillioides*	Inhibition of growth of *Fusarium verticillioides*	R-myclobutanil more active than S-myclobutanil	Li et al. 2018
Herbicide	Lactofen	*Echinochloa crusgali*	Inhibition of growth of *Echinochloa crusgali*	R-lactofen was more active than S-lactofen	Xie et al. 2018
Fungicide	Epoxiconazole	*Chlorella vulgaris*	Inhibition of growth of *Chlorella vulgaris*	S,R-epoxiconazole was more active than R,S-epoxiconazole	Kaziem et al. 2020
Herbicide	Imazapyr	*Aradidopsis thalina*	Inhibition of chlorophyll synthesis	(+)-imazapyr was more active than (-)-imazapyr	Hsiao, Wang, and Yen 2014
Herbicide	Napropamide	*Poa annua*	Inhibition of growth of *Poa annua*	(-)-naprpamide was more active than (+)-napropamide	Qi et al. 2015

Triazole fungicide	Flutriafol	*Rhizoctonia solani*, *Alternaria solani*, *Pyricularia grisea*, *Gibberella zeae* and *Botrytis cinerea*	Inhibition of growth of fungi	R-flutriafol was more active than S-flutriafol	Zhang et al. 2015

6. Enantioselective toxicity of chiral pesticides on non-target organisms

Enantiomers of chiral pesticides are known to exhibit selective interaction with biological systems (Tong et al. 2019). Usually one of the enantiomers, as already alluded to, show pronounced bioactivity to the target species in a chiral environment (Birolli et al. 2019). The other enantiomer will be inactive or less active to the target species and, therefore, is discharged into the environment in relatively large quantities where it may cause adverse effects to non-target fauna and flora. The toxicity of chiral pesticides to no-target organisms is, therefore, usually enantioselective (Birolli et al. 2019; Buerge et al. 2019; Duan et al. 2018; Fonseca et al. 2019; Kuhlmann et al. 2019; Qu et al. 2016; Tong et al. 2019; Xiang et al. 2019) (Table 5).

Toxicity of phenylpyrazole pesticides (fipronil, flufiprole and ethiprol) on non-target organisms have been extensively studied by many researchers (Qian et al. 2019; Qu et al. 2016; Zhan et al. 2018; Gao et al. 2020; Xu et al. 2019; S. Wang, Li, and You 2019) (Table 5). Their results showed that these pesticides have enantioselective toxicity to non-target organisms, in most cases. For instance, Qian et al. (2019) studied the toxicity of fipronil on zebrafish, a non-target organism and established that it was enantioselective. They reported that R-fipronil caused more severe anxiety-like behaviour, such as increased swimming speed and dysregulated photoperiodic locomotion as well as increased neurotoxicity to embryonic and larval zebrafish as compared with S-fipronil. In a similar study, Qin et al. (2015) investigated the toxicity of fipronil on *Eisenia foetida* earthworm and the results showed that the R-enantiomer was more toxic to these non-target organisms than the S-form. Qu et al. (2016) studied the enantioselective toxicity of fipronil to non-traget organisms *Lemna minor* and *Anodonta woodiana* found in aquatic ecosystems. Their findings indicated that S-fipronil was more toxic than R-fipronil against *Anodonta woodiana*, while R-fipronil was more toxic for *Lemna minor*. Gao et al. (2020) investigated the toxicity of flufiprole to *Chlorella pyrenoidosa*. The results showed that flufiprole exhibited enantioselective toxicity towards *Chlorella pyrenoidosa*. R-flufiprole exhibited greater toxicity to *Chlorella pyrenoidosa* than the S-enantiomer. In a similar study, Gao et al. (2019) studied the enantioselective toxicity of the enantiomers of ethiprole to *Chlorella pyrenoidosa*. The results indicated that R-ethiprole was more toxic to non-target *Chlorella pyrenoidosa* than S-ethiprole.

Pyrethroid pesticides are among the most used agrochemicals for pest control (Brander et al. 2016). Most of them are chiral and highly lipophilic, and have a high tendency to bio-accumulate in living organisms. These chiral pesticides have endocrine disrupting effects in organisms and they exhibit enantioselective toxicity to non-target organisms. Some researchers have studied the enantioselective toxicity of chiral pyrethroids on non-target organisms. For instance, Xiang et al. (2019) investigated the toxicity of the enantiomers of cis-bifenthrin on the reproduction of non-target zebrafish. The results indicated that S-cis-bifenthrin caused more severe reproductive endocrine disturbance in zebrafish than R-cis-bifenthrin. In a similar study, Zhang et al. (2019) studied the enantioselective toxicity of cis-bifenthrin on non-target *Xenopus laevis*. Their findings indicated that R-cis-bifenthrin caused serious negative effects on the behaviour and development of *Xenopus laevis* than S-cis-bifenthrin. Thus, cis-bifenthrin has enantioselective toxicity on *Xenopus laevis*. In the same vein, Xiang et al. (2018) investigated enantioselective effects of cis-bifenthrin on lipogenesis in human hepatoma cells. Their results indicated that S-cis-bifenthrin triggered rapid lipid metabolism in human hepatoma cells causing high prevalence of obesity. On the other hand, Diao et al. (2011) investigated the toxicity of the enantiomers of alpha-cypermethrin on non-target earthworm *Eisenia fetida*. They found out that (+)-(*1R-cis-αS*)-alpha-cypermethrin was 30 times more toxic than the (−)-(*1S-cis-αR*)-isomer as it caused increased mortality of the earthworm.

Many researchers have shown tremendous interest in the enantioselective toxicity of chiral herbicides (Chen et al. 2019; Duan et al. 2018; Xu et al. 2019; Wang et al. 2018; Zhang et al. 2019) (Table 5). The general observation made by researchers was that the toxicity of most chiral herbicides on non-target organisms was enantioselective in nature. For instance, Xie et al. (2018) investigated the toxicity of the enantiomers of lactofen on non-target *Microcysts aeruginosa*. They found out that S-lactofen was more potent towards *Microcysts aeruginosa* than R-lactofen. In a similar study, Wang et al. (2018) studied enantioselective toxicity of lactofen on non-target zebrafish *Danio rerio*. They obtained similar results where the S-lactofen was more toxic towards zebrafish *Danio rerio* than R-form. On the other hand, Jing et al. (2017) performed a research on the toxicity of the enantiomers of fenoxaprop-ethyl herbicide on non-target tadpoles. They reported that S-fenoxaprop-ethyl was more toxic to the tadpoles than R-fenoxaprop-ethyl. The investigation carried out by Zhang et al. (2019) on the toxicity of the enantiomers of glufosinate herbicide on non-target maize crop also showed enantioselectivity. They reported that S-glufosinate caused severe reduction in shoot growth of maize as compared to R-glufosinate.

Another class of chiral pesticides that is used commonly for both domestic and industrial purposes is the fungicide. Most fungicides have stereogenic centres and exhibit enantioselective toxicity to non-target organisms. For instance, Kaziem et al. (2020) investigated the toxicity of the enantiomers of epoxiconazole on non-target *Daphnia magna*. The results indicated that R,S-epoxiconazole was more potent than S,R-epoxiconazole. Thus, epoxiconazole had enantioselective toxicity towards *Daphnia magna*. Zhai et al. (2019) investigated the enantioselective toxicity of prothioconazole on non-target *Lemna minor*, *Chlorella pyrenoidosa* and *Daphnia magna*. They reported that the toxicity of (+)-prothioconazole was higher than that of (-)-prothioconazole on *Lemna minor* and *Chlorella pyrenoidosa* while (-)-prothioconazole was more potent on *Daphnia magna* than (+)-prothioconazole.

Table 5: Enantioselective toxicity of the enantiomers of chiral pesticides on non-target organisms

Class of chiral pesticide	Chiral pesticide studied	Non-target organism	Toxicity on non-target organism	Toxic enantiomer	Reference
Pyrethroid	Cis-bifenthrin	*Xenopus laevis*	Adverse effect on behaviour and development of *Xenopus laevis*	R-cis-bifenthrin is more toxic than S-cis-bifenthrin	Zhang et al. 2019
Herbicide	Acetochloro	Zebrefish embryo-larvae	Disruption of thyroid gland activities	S-acetochloro is more potent than R-acetochloro	Xu et al. 2019
Pyrethroid	Cis-bifenthrin	Humans	Triggering development of obesity in humans	S-cis-bifenthrin triggers rapid lipid metabolism in human hepatoma cells than the R-form	Xiang et al. 2018
Herbicide	Glufosinate	Maize	Reduction in growth rate, shoot height and shoot weight	S-glufosinate is more toxic than R-glufosinate	Zhang et al. 2019

Pyrethroid	Cis-bifenthrin	Zebrafish	Damage of the reproductive system of zebrafish	S-cis-bifenthrin caused more severe damage to the reproductive system of zebrafish than R-cis-bifenthrin	Zhang et al. 2019
Herbicide	Dichlorprop	*Arabidopsis thaliana*	Disturbance of fatty acid synthesis	R-dichlorprop more toxic than S-dichlorprop	Chen et al. 2019
Pyrethroid	Alpha-cypermethrin	Earthworm *Eisenia fetida*	Increased mortality rate of the earthworm	1R-cis-αS enantiomer more toxic	Diao et al. 2011
Herbicide	Lactofen	Zebrafish *Danio rerio*	Cause genetic defects in zebrafish *Danio rerio*	S-lactofen more potent than R-lactofen	Wang et al. 2018
Fungicide	Epoxiconazole	*Daphnia magna*	Increased death of *Daphnia magna*	R,S-epoxiconazole more toxic than S,R-epoxiconazole	Kaziem et al. 2020
Herbicide	Cartentrazone-ethyl	Maize	Inhibition of radicle growth	S-cartentrazone-ethyl more toxic than R-cartentrazone-ethyl	Duan et al. 2018

Fungicide	Imazalil	*Daphnia magna*	Increased death of *Daphnia magna*	S-imazalil was 2.25-fold more toxic than R-imazalil	Li et al. 2019
Herbicide	Lactofen	*Microcysts aeruginosa*	Inhibition of algal growth in aquatic environments	S-lactofen more potent than R-lactofen	Xie et al. 2018
Herbicide	Fenoxaprop-ethyl	Tadpoles	Increased mortality of tadpoles	S-fenoxaprop-ethyl more potent than R-fenoxaprop-ethyl	Jing et al. 2017
Fungicide	Prothioconazole	*Lemna minor* and *Chlorella pyrenoidosa*	Increased mortality of *Lemna minor* and *Chlorella pyrenoidosa*	(+)-prothioconazole more toxic than (-)-prothioconazole	Zhai et al. 2019
Fungicide	Prothioconazole	*Daphnia magna*	Increased mortality of *Daphnia magna*	(-)-prothioconazole more toxic than (+)-prothioconazole	Zhai et al. 2019

7. Pre-concentration of chiral pesticides

Chiral pesticides can have detrimental effects on the environment even at trace concentrations. Thus, effective pre-concentration techniques are required to enable their monitoring in the environment at these trace levels. There are so many pre-concentration techniques that have been employed by researchers during chiral analysis by chromatographic techniques. These include QuEChERS (Jiang et al. 2018; Zhang et al. 2018; He et al. 2018; Shi et al. 2018), SPE (Zhang et al. 2019; Gao et al. 2016), SPME (Dallegrave et al. 2016), MSPE (Zhao et al. 2019) and liquid extraction (de Albuquerque, de Matos, and de Oliveira 2016; Xiang et al. 2019; Zhang et al. 2018) (Table 6 - 8).

The QuEChERS technique is widely used during the pre-concentration of chiral pesticides prior to chromatographic analysis (Tables 6 - 8). Its popularity can be attributed to its simplicity, environmental friendliness and robustness during pre-concentration of chiral pesticides. SPME and MSPE, just like QuEChERS, are miniaturised techniques with very low environmental footprints when used to pre-concentrate chiral pesticides. Thus, they are also used by environmentally conscious researchers for pre-concentration of chiral pesticides in different matrices. SPE and liquid-liquid extraction, although they use large volumes of toxic organic solvents, are equally popular techniques for pre-concentrating chiral pesticides (Table 6 - 8). Their popularity probably stems from the fact that most laboratories have equipment used to perform these techniques. In addition, this may be due to resistance to change the old mind-set among those researchers who have used these techniques for a long time and inability to embrace new eco-friendly methods that can be used for pre-concentration with greater efficiency.

8. Chiral separation techniques

8.1. Chiral high performance liquid chromatography

High performance liquid chromatography (HPLC) is one of the most versatile and popular analytical techniques used during chiral analysis of pesticides (Díaz Merino et al. 2019; Gao et al. 2016; Zhang et al. 2019) (Table 6). The popularity of HPLC hinges on the fact that it is a rapid and a non-destructive technique. It is regarded as the best technique for the preparation and separation of enantiopure chemicals such chiral pesticides. For instance, Cui et al. (2018) successfully separated the enantiomers of tebuconazole using HPLC with a chiralpak 1C column. The chromatogram they obtained is shown in Fig. 6a. From the chromatogram, it can be observed that they managed to achieve baseline resolution of the enantiomers of tebuconazole. Gao et al. (2016), in the same vein, separated the enantiomers of isofenphos-methyl also using HPLC with a lux cellulose-3 column. Their chromatogram (Fig. 6b) also show good baseline resolution of the enantiomers of isofenphos-methyl. Thus, HPLC is a versatile and useful technique for the separation of the enantiomers of chiral pesticides.

Enantiomers of chiral pesticides cannot be separated in achiral environment. Thus, the success of the HPLC technique during chiral separation depends on the availability of efficient chiral stationary phases for effective chiral recognition. Many researchers have used chiral stationary phases derived from cellulose during analysis of chiral pesticides (Table 6). For instance, Zhang et al. (2019) successfully separated the enantiomers of triazole fungicide called penconazole using ultra performance liquid chromatographic technique with a lux cellulose-2 column as a chiral stationary phase. A mixture of 0.1% formic acid in methanol and 10 mmol L^{-1} ammonium acetate in water (75/25, v/v) was used as the mobile phase. Enantioselective elution of the components of the racemic mixture was observed during this chromatographic technique. The enantiomer (+)-penconazole was firstly eluted followed by (-)-penconazole. The method showed reliable performances in linearity, recovery and precision. In the same vein, Liu and Ding (2019) performed a simultaneous enantiomeric separation of prothioconazole and prothioconazole-destho using lux cellulose-1 column as a chiral stationary phase of HPLC. A mixture of acetonitrile and water was used as mobile phase and good linearity and recoveries were obtained. Wang et al. (2020) used the same column as a stationary phase during the separation of the enantiomers of triadimenol from *Eremias argus* lizard tissues using LC-MS/MS. Tissue distribution experiments showed enantioselective biotransformation of the triadimenol in the lizards.

Other researchers used amylose-based chiral stationary phases during chiral separation using HPLC (Table 6). For instance, Díaz Merino et al. (2019) used silica-based column with immobilised amylose tris(3-chloro-5-methylphenycrbamates) (chilalpak IG-3) as a stationary phase during chiral separation of thirty multiclass pesticides. Good resolution of the enantiomers of these pesticides was obtained. Qi et al. (2015) compared enantioseparation selectivity of isocarbophos enantiomers using four cellulose-based chiral columns and two amylose-based columns. The results showed that the fastest baseline separation of isocarbophos was obtained when amylose tris(3,5-dimethylphenylcarbamate) (chiralpak AD-3R) was used as the stationary phase. In the same vein, Chai et al. (2013) compared the resolution of cellulose-based stationary phase with the amylose-based stationary phase. It was observed that baseline separations were obtained with phenomene lux cellulose-1, lux cellulose-2 and lux amylose-2, using 2-propanol/hexane as the mobile phase. Li et al. (2013) used an amylose-based stationary phase (chiralpak AD-RH) for the enantioseparation of nine multiclass pesticides from soil and water samples. The mean recoveries for all the enantiomers were above 77.8% and good linearity (r^2 = 0.9986) was obtained. Thus, amylose-based chiral stationary phases usually have effective chiral recognition sites for most chiral pesticides.

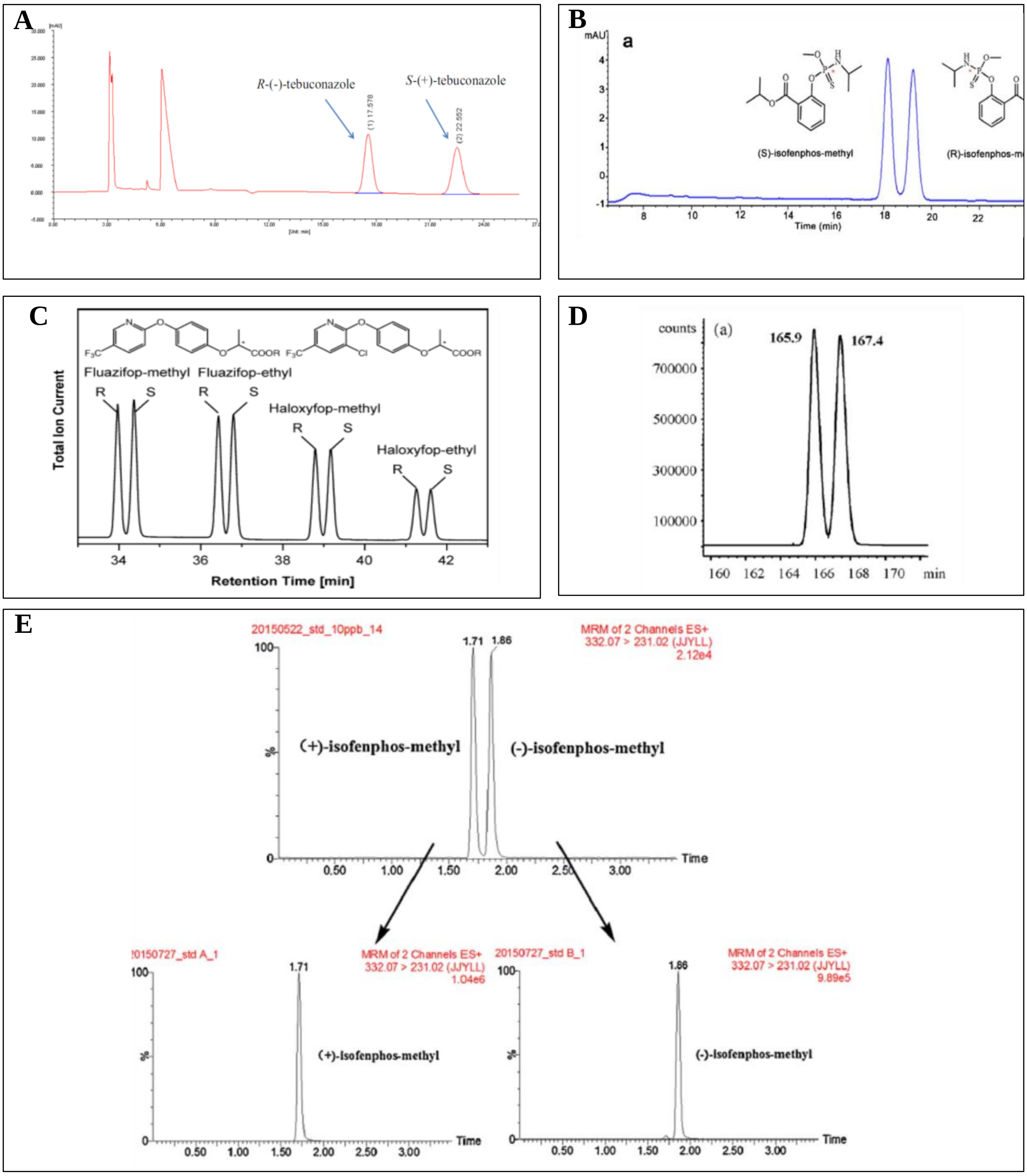


Fig. 6: Chromatogram for the separation of (a) tebuconazole enantiomers by HPLC-DAD (Cui et al. 2018) (b) isofenphos enantiomers by HPLC-UV (Gao et al. 2016) (c) enantiomers of fluazifop-methyl, fluazifop-ethyl, haloxyfop-methyl and haloxyfop-ethyl by GC-MS (Buerge et al. 2015) (d) enantiomers of bromocyclen by GC-ECD (Fidalgo-Used et al. 2008) and (e) enantiomers of isofenphos by SFC-MS/MS (Chen et al. 2016)

Table 6: Analysis of chiral pesticides using enantioselective high performance liquid chromatography

Matrix	Class of pesticide(s)	Chiral pesticide(s)	Extraction technique	Chiral stationary phase column	Detector	Reference
Panax notogiseng, lyceum, barbarum, radixastragali, chrysanthemum, rose and lily	Multi-class pesticides	Novaluron, triapenthenol, etoxazole, bromacil, surecide and fenarimol	MSPE	Chiralpak 1G column	ng	Zhao et al. 2019
Cucumber	Carbamate	Fenobucarb	Ng	Daicel IG-3 chiral column	UPLC-MS/MS	Xia et al. 2019
Grape, tea, lotus root, lotus leaf, lotus seeds and hulls	Triazole fungicide	Penconazole	Graphite carbon black SPE	Lux cellulose-2 column	MS/MS	Zhang et al. 2019
Eremias argus lizard	Fungicide	Triadimenol	Liquid extraction	Lux cellulose-1 column	MS/MS	Wang et al. 2019

Environmental matrices	Multiclass pesticides	30 chiral pesticides	Ng	Chiralpak IG-3	DAD	Díaz Merino et al. 2019
Soil and water	Pyrethroids	Bifenthrin and lambda-cyhalothrin	Liquiq-liquid extraction	Lux cellulose-1 column, luk-cellulose-3 column and chiralpak columns	DAD	Zhang et al. 2018
Water	Herbicides	Lactofan	Ng	Chiralcel AD-H column	PDA	Xie et al. 2018
Food and environmental matrices	Triazole fungicide	Metconazole	QuEChERS	Enantiopak-OD column	UV	He et al. 2018
Corn and corn plant	α-aminophosphate	Dufulin	QuEChERS	Chiralpak IC column	MS/MS	Shi et al. 2018
Soils	Pyrethroids	Fenpropathrin	Ng	Lux cellulose-3 column	ng	P. Zhang et al. 2017
Water	Phenylpyrazole	Fipronil, flufiprole and ethiprole	Ng	Chiralpak IB column	DAD	J. Gao et al. 2017

Water	Herbicides	Acetochlor, metolachlor, propisochlor and napropamide	Ng	Chiralpak AS-H, OD-H and AY-H columns	DAD	J. Xie et al. 2016
Human liver microsomes	Nematocide	Fenamiphos	Liquid-liquid extraction	Chiralpak AS-H column	ng	de Albuquerque, de Matos, and de Oliveira 2016
Fruits, vegetables and soils	Organophosphates	Isofenphos-methyl	SPE	Lux cellulose-3 column	UV	Gao et al. 2016
Orange pulp. Orange peel and kumquate	Organophosphates	Isocarbiphos	QuEChERS	Chiralpak AD-3R	MS/MS	Qi et al. 2015

Note; ng – not given

8.2. Chiral gas chromatography

Chiral gas chromatography is a chromatographic technique that can be used for the separation of the enantiomers of chiral compounds. Many researchers have used chiral GC for the enantioseparation of chiral pesticides from different complex matrices (Xiang et al. 2019; Yao et al. 2019; Buerge et al. 2016; Kalathoor et al. 2015; Dallegrave et al. 2016) (Table 7). Although chiral GC is a versatile analytical technique, it cannot be used to separate the enantiomers of all chiral pesticides. This technique can only separate the enantiomers of chiral pesticides that are volatile. The enantiomers of the chiral pesticides must easily vaporise within the temperature range used in the chiral GC oven without decomposing.

Researchers, using chiral GC, have managed to separate volatile enantiomers of many different classes of chiral pesticides. These include pyrethroids (Xiang et al. 2019; Yao et al. 2019; Corcellas, Eljarrat, and Barceló 2015), fungicides (Kalathoor et al. 2015; Buerge et al. 2016) and herbicides (Buerge et al. 2015) (Table 7). Generally, good resolution of the enantiomers was obtained when volatile chiral pesticides were subjected to chiral GC analysis. For instance, Buerge et al. (2015) separated the enantiomers of four herbicides, namely fluazifop methyl, fluazifop ethyl, haloxyfop methyl and haloxyfop ethyl, using chiral GC. The chromatogram they obtained is shown in Fig. 6c and it clearly shows that they managed to achieve baseline resolution of the enantiomers of the herbicides used. In the same vein, Fidalgo-Used et al. (2008) separated enantiomers of bromocyclen using chiral GC. Good resolution of the enantiomers was achieved as indicated in the chromatogram (Fig. 6d) they obtained.

During chiral GC analysis, a carrier gas is used as the mobile phase. The carrier gas is usually an inert gas such as helium or an unreactive gas such as nitrogen. Many researchers have used helium as a carrier gas during the separation of the enantiomers of chiral pesticides by chiral GC (Corcellas, Eljarrat, and Barceló 2015; Kalathoor et al. 2015; Xiang et al. 2019). For instance, Kalathoor et al. (2015) achieved baseline separation of the enantiomers of metalaxyl using helium as a mobile phase. In a similar study, Corcellas, Eljarrat, and Barceló (2015) managed to effectively separate enantiomers of several pyrethroids also using helium as the mobile phase. Some researchers, however, used nitrogen as the mobile phase during the separation of the enantiomers of chiral pesticides by GC. For example, Yao et al. (2019) obtained good resolution of the enantiomers of alpha-cypermethrin using nitrogen as the mobile phase.

Effective chiral stationary phases are required during separation of the enantiomers of chiral pesticides by chiral GC. Many commercial chiral stationary phases are available on the market and many researchers have used them for the enantioseparation of chiral pesticides using GC (Naudé and Rohwer 2012; Buerge et al. 2016; Yao et al. 2019; Xiang et al. 2019) (Table 7). For instance, Kalathoo et al. (2015) used the BGB-172 chiral column during the separation of the enantiomersa of metalaxyl from soil samples by chiral GC. A good resolution of 0.95 was obtained for the enantiomers. Corcellas et al. (2015) used the same column, in similar study, for the enantioseparation of several chiral pesticides in breast milk. Resolutions greater than 0.58 were obtained for all the enantiomers of the chiral pesticides used. On the other hand, Dallegrave et al. (2016) used DB-5MS chiral column during separation of the enantiomers of seventeen pyrethroids in different complex matrices with good resolution of the enantiomers. Another chiral stationary phase that have been used by some researchers during enantioseparation of asymmetric pesticides by GC is the HP-5 column. For instance, Xiang et al. (2019) successfully achieved baseline separation of the enantiomers of bifenthrin using the HP-5 chiral column.

Table 7: Analysis of chiral pesticides using enantioselective gas chromatography

Matrix	Class of chiral pesticide(s)	Chiral pesticide(s)	Extraction technique	Chiral stationary phase column	Detector	Reference
Zebra fish	Pyrethroid	Bifenthrin	Liquid-liquid extraction	HP-5 column	ECD	Xiang et al. 2019
Tomato, cucumber, rape, cabbage and pepper	Pyrethroid	α-cypermethrin	Liquid-liquid extraction	HP-5 column	ECD	Yao et al. 2019
Beef, chicken, eggs, fish and milk	Pyrethroid	17 pyrethroids	Ultrasound extraction and SPME	DB-5MS capillary column	MS/MS	Dallegrave et al. 2016
Grapevines, sugar beets and wheat	Fungicides	Fenpropidin, fenpropimorph and spiroxamine	QuEChERS	BGB-5 column	MS/MS	Buerge et al. 2016

Blackgrass and garden cress	Herbicides	Haloxyfop-methyl	Sonication	BGB column coated with permethyl β-cyclodextrin	MS	Buerge et al. 2015
Soil	Fungicides	Metalaxyl	Ultrasonication	BGB-172 column	MS	Kalathoor et al. 2015
Human breast milk	Pyrethroids	Bifenthrin, cyhalothrin, cyfluthrin, cypermethrin, permethrin and tetramethrin	Sonication	BGB-172 column	MS/MS	Corcellas, Eljarrat, and Barceló 2015
Soil and air	Organochlorine	DDT	Ng	β-cyclodextrin-based BGB-172 column	GC x GC-TOF-MS	Naudé and Rohwer 2012

Note: ng – not given

8.3. Chiral supercritical fluid liquid chromatography

Supercritical fluid chromatography (SFC) is among the chromatographic techniques that are preferred during the enantioselective separations and purifications of chiral pesticides (Tao et al. 2018; Jiang et al. 2018) (Table 8). The mobile phase used during SFC is usually supercritical CO_2 (Liu et al. 2015; Pan et al. 2016). Polar organic modifiers or co-solvents are usually added to the supercritical CO_2 in order to enhance its elution strength of the chiral pesticides (Zhang et al. 2018; Tan et al. 2017). Alcohols such as propanol, ethanol and methanol are the commonly used co-solvents during SFC (Table 8). The physico-chemical properties of supercritical CO_2 such as lower viscosity and higher diffusivity give it an edge over the mobile phases used in other chromatographic techniques (Tao et al. 2018). Thus, SFC has higher throughput and higher flow rates as compared to HPLC and usually has good resolution of the enantiomers of chiral pesticides (Fig 6e). Consequently, SFC has shorter analysis time and faster column equilibration during analysis of chiral pesticides (Liu et al. 2015). SFC is also considered an environment-friendly method due to reduced consumption of toxic solvents and additives. Additional advantages are that CO_2 is characterized as cheap, non-flammable and non-toxic as compared to organic solvents (Pan et al. 2016; Tao et al. 2018).

Chiral triazole fungicides are among the pesticides that have been extensively studied and analysed by many researchers using SFC (Jiang et al. 2018; He et al. 2016; Pan et al. 2016; Tan et al. 2017; Tao et al. 2018) (Table 8). During the separation of the enantiomers of chiral triazole fungicides with SFC, some chemical modifiers are added to the supercritical CO_2. Triazole fungicides are polar compounds while the mobile phase, supercritical CO_2, is non-polar. Thus, polar chemical modifiers are added to the mobile phase, as already alluded to, for the enhancement of the elution of the polar triazole fungicide molecules. For instance, Jiang et al. (2018) added 2-propanol to the supercritical CO_2 mobile phase during chiral separation of the enantiomers of prothioconazole using SFC. Tan et al. (2017) used ethanol has a chemical modifier of supercritical CO_2 mobile phase during enantioseparation of triticonazole using SFC. In the same vein, Pan et al. (2016) combined supercritical CO_2 with methanol as a co-solvent during the SFC-based chiral separation of enantiomers of pyrisoxazole. Thus, alcohols are usually used as chemical modifiers during SFC and their popularity hinges largely on the fact that they are relatively inexpensive and easily accessible polar solvents.

SFC separation of the enantiomers of chiral pesticides often involves their direct resolution using columns packed with chiral stationary phases. So many commercial chiral stationary phases are on the market and have been used by researchers for enantioseparation of chiral pesticides (He et al. 2016; Pan et al. 2016; Jiang et al. 2018) (Table 7). For instance, Jiang et al. (2018) used enantiopak OD column for the separation of the enantiomers of prothioconazole in tomato and soil samples using SFC. The enantiomers were baseline separated with a resolution of 3.59. Tan et al. (2017) also successfully used enantiopak OD column for chiral separation of the enantiomers of triticonazole in tomato and cucumber using SFC. Baseline separation of the enantiomers of triticonazole was achieved using this column. In the same vein, He et al. (2016) also obtained good resolution of the enantiomers of triticonazole using SFC and the same column. Other researchers successfully separated the enantiomers of chiral pesticides using SFC with the chiralpak AD-3 column. For instance, Cheng et al. (2017) successfully resolved enantiomers of propiconazole from wheat, grapes and soil using SFC with a chiralpak AD-3 column. In a similar study, Zhang, Miao, and Lin (2018) also separated the enantiomers of organophosphate pesticides using SFC with the chiralpak AD-3 column. Other columns used by researchers for enantioseparation of chiral pesticides using SFC include chiralpak IA column (Pan et al. 2016), trefoil AMY 1 column (Liu et al. 2016) and chiral CCA column (Zhang et al. 2018).

Table 8: Analysis of chiral pesticides using enantioselective supercritical fluid chromatography

Matrix	Class of pesticide(s)	Chiral pesticide(s)	Extraction technique	Mobile phase	Chiral stationary phase column	Detector	Reference
Tomato and soil	Triazole fungicide	Prothioconazole	QuEChERS	CO_2/ 2-propanol	EnantioPax OD column	VCD spectroscopy	Jiang et al. 2018
Tea, apple and grapes	Triazole fungicide	Diniconazole	Liquid extraction	CO_2/ isopropanol	Chiral CCA column	Q-TOF/MS	Zhang et al. 2018
Environmental water	Organophosphates and phenylpyrazole pesticides	Isofenphos, isophenyl-methyl, isocarbophos, flufiprole, fipronil and ethiprole.	Ng	CO_2	Chiralpax AD-3	ng	Zhang, Miao, and Lin 2018
Fruits, vegetables, cereals and soil	Triazole fungicide	Fenbuconazole	QuEChERS	CO_2/ ethanol	ACQUITY UPC2 Trefoil AMY 1 column	Tandem mass spectrometer	Tao et al. 2018

Tomatoes and cucumber	Triazole fungicide	Triticonazole	QuEChERS	CO_2/ ethanol	EnantioPax OD column	UV	Tan et al. 2017
Wheat, grapes and soil	Triazole fungicide	Propiconazole	QuEChERS	CO_2/ ethanol	Chiralpak AD-3 column	Tandem mass spectrometer	Cheng et al. 2017
Aqueous solutions	Triazole fungicide	Triticonazole	Ng	CO_2/ ethanol	EnantioPax OD column	PDA	He et al. 2016
Cucumber, tomato and soil	Fungicide	Pyrisoxazole	QuEChERS	CO_2/ methanol	Chiralpak IA column	Tandem mass spectrometer	Pan et al. 2016
Soil	Acaricide	Cyflumetofen	QuEChERS	CO_2/ methanol	Trefoil AMY 1	Tandem mass spectrometer	Liu et al. 2016
Water and zebrafish	Triazole fungicide	Tebuconzole	QuEChERS	CO_2/ methanol	Chiralpak IA-3	Tandem mass spectrometer	Liu et al. 2015

Note: ng – not given; Q-TOF/MS - quadrupole time-of-flight mass spectrometry; VCD - vibrational circular dichroism

9. Future prospects and challenges

Chiral pesticides have contributed tremendously towards sustainable food security in the world. Their use, however, is not free of challenges. Most chiral pesticides that are on the market right now are sold as racemates and the enantiomers, as already alluded to, have enantioselective toxicity to the target organisms. One enantiomer, in most cases, will be active to the target organism and the other ones will be inactive. Thus, the accumulation of the inactive enantiomers in the environment is a serious environmental challenge. This entails that the analysis of the enantiomers of chiral pesticides in the environment is imperative. The pre-concentration and analysis of chiral pesticides, however, is hampered by the lack of enantiopure standards that are easy to synthesis and purify. This, therefore, has a negative impact on the quantification of the enantiomers of the chiral pesticides in different environmental matrices. Synthesis of enantiopure chiral pesticides seems to be the most attractive and plausible step in minimising the choking of the environment with the inactive enantiomers of the chiral pesticides. This would ensure that only the active enantiomer would be applied to crops during pest control. Although this seems to be a better strategy, it is still far from providing a permanent solution to the environmental problems associated with chiral pesticides. This is due to the fact that some chiral pesticides have labile asymmetric centres and enantiomers may undergo stereo-chemical inversion. This process of enantiomerization complicates control of pollution of the environment by chiral pesticides as well as their analysis. During chromatographic analysis of chiral pesticides, the stationary phases have chiral recognition sites that are specific to one of the enantiomers. Chiral inversion, therefore, makes it difficult to quantify accurately the target enantiomer through chromatographic analysis. Mitigation of pollution of the environment by chiral pesticides lies in minimisation of their use during pest control and, where possible, to resort to the use of bio-based chiral pesticides that are not persistent in the environment and are biodegradable.

10. Conclusion

A significant number of the pesticides used for the mitigation of the detrimental effects of pests on crops are chiral. Some of these chiral pesticides are subsequently released into the environment where they exhibit enantioselective activity and toxicity to target and non-target organisms, respectively. Due to their toxicity to non-target organisms, the separation and analysis of the inactive enantiomers of chiral pesticides is mandatory. Thus, chiral analysis is a powerful and necessary tool for studying the behaviour of chiral pesticides in the environment. Chiral analysis of pesticides, in recent years, has been dominated by chromatographic techniques. The commonly used enantioselective chromatographic techniques include gas chromatography, high performance liquid chromatography and supercritical fluid chromatography. Good resolution of the enantiomers during these chromatographic techniques hinges on the correct choice of the chiral stationary phase. The most popular chiral stationary phases employed during separation of asymmetric pesticides are those that are polysaccharide-based. Cellulose and amylose are among the commonly used polysaccharides during the synthesis of chiral stationary phases. They have proved to have versatile enantioselective chiral recognition sites during chromatographic analysis of asymmetric pesticides.

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Conflicts of Interest

The authors declare no conflict of interest.

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