IDENTIFICATION OF AGRICULTURAL AND INDUSTRIAL POLLUTANTS IN THE KAT RIVER, EASTERN CAPE AND THEIR EFFECT ON AGRICULTURAL PRODUCTS FOUND ALONG THE RIVER BANKS

A Dissertation

Submitted in fulfilment of the requirements for the degree of
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BY

NHAMO MUTINGWENDE

SUPERVISOR: PROF G. BRADLEY

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I Nhamo Mutingwende declare that this dissertation titled “Identification of agricultural and industrial pollutants in the Kat River, Eastern Cape and their effect on agricultural products and human settlements found on the river banks” submitted for the award of the Master of Science degree in Biochemistry at the University of Fort Hare, is my original work with exemption to the citations. This work has not been submitted at any other University in partial or entirety for the award of any degree.

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DEDICATION

I want to dedicate this Thesis to my mother Elizabeth Mutingwende. “Mom thank you so much for allowing me the privilege to be an educated son to you when you couldn’t have the privilege. You are the reason why I am this far in my education. You couldn’t have an opportunity to see the door of a classroom, not because it was your intention, but because of circumstances beyond your control. Growing up wasn’t all that easy but thank you for nurturing me and giving me the love I needed to move forward. I could only be stronger. Because of you I have set a record in the family. I did it for you. It’s for you Mom”.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF EQUATIONS

LIST OF ANNEXTURES

ACRONYMS

AOAC	Ethanolic
APCI	Atmospheric Pressure Chemical lonisation
BOD	Biochemical Oxygen Demand
CE	Collision Energy
CES	Collision Energy Spread
CO_2	Carbon Dioxide
CWA	Clean Water Act
DDD	Dichlorodiphenyltrichloroethane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DOC	Dissolved Organic Carbon
DP	Declustering Potential
EDC	Endocrine Disrupting Compounds
EMEA	European Medicines Evaluation Agency
EPA	Environmental Protection Agency, (Irish)
ERA	Environmental Risk Assessment
ESI	ElectroSpray lonisation
EU	European Union
F&V	Fruit and Vegetable
FAO	Food and Agriculture Organization
FDCA	Food, Drug, and Cosmetics Act

FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FQPA	Food Quality Protection Act
GS1	Ion Source Gas 1
GS2	Ion Source Gas 2
H	Henry's Constant
H_2O	Water
HPLC	High Performance Liquid Chromatography
IDA	Independent Data Acquisition
ISVF	Ion Spray Voltage Floating parameter
KD	Distribution Coefficient
K_{OC}	Soil Sorption Coefficient
K_{OW}	Water Partition Coefficient
LC/MS	Liquid Chromatograph Mass Spectrometer
LC/MS/MS	Liquid Chromatography Mass Spectrometry, (tandem-MS)
LD50	Lethal Dose (50%)
MCLs	Maximum Contaminant Levels
MRLs	Maximum Residue Limits
MRM	Multiple Reaction Monitoring
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
OPs	Organophosphates
PAPS	3'phosphoadenosine-5'-phosphosulphate
PBT	Bio Accumulative, and Toxic
PD	Parkinson’s Disease

POEA	Polyethylene Amine
PPCPs	Pesticides and Pharmaceuticals and Personal Care Products
QuEChERS	Quick Easy Cheap Effective Rugged and Safe
REI	Restricted-Entry Interval
SDWA	Safe Drinking Water Act
STP	Sewage Treatment Plants
TEM	Temperature
TMDLs	Total Maximum Daily Loads
TOF	Time of Flight
UDP	Uridine Diphosphate
WQS	Water Quality Standards
WWII	World War II
WWTP	Waste Water Treatment Plant
XIC	Extracted Ion Chromatogram

Abstract

There is growing concern that commonly used Pharmaceuticals and Personal Care Products (PPCPs) and pesticides are entering and contaminating drinking water supplies. The use of targeted quantitation of PPCP has been well established but there is an emerging trend to also screen for and identify unexpected environmental pollutants. Chemicals like pesticides hormones and antibiotics are especially of interest because of proven endocrine disrupting effects and a possible development of bacterial resistance. Powerful screening methods are required to detect and quantify the presence of these compounds in our environment. PPCP encompass a wide range of pollutants, including Endocrine Disrupting Compounds (EDC), pesticides, hormones, antibiotics, drugs of abuse, x-ray contrast agents and drinking water disinfection by-products to name a few. In order to properly assess the effects of these compounds on our environment, it is necessary to accurately monitor their presence. The diversity of chemical properties of these compounds makes method development challenging. LC/MS/MS is able to analyse polar, semi-volatile, and thermally labile compounds covering a wide molecular weight range.

The new AB SCIEX TripleTOF™5600 LC/MS/MS was used to profile environmental samples for unexpected pollutants, to identify and characterise the chemical composition and structure of the pollutants, and to quantify (based on intensity) the concentration in collected water samples. Liquid Chromatography coupled to tandem Mass Spectrometry (LCMS/ MS) is able to analyse polar, semi-volatile, and thermally labile compounds covering a wide molecular weight range, such as pesticides, antibiotics, drugs of abuse, x-ray contrast agents, drinking water disinfection by-products etc. More recently there is a growing interest from environmental researchers to also screen for and identify non-targeted compounds in environmental samples, including metabolites and degradates, but also completely unexpected pollutants. The new AB SCIEX TripleTOF™5600 LC/MS/MS system is capable of performing highly sensitive and fast MS scanning experiments to search for unknown molecular ions while also performing selective and characteristic MS/MS scanning for further compound identification and, therefore, is the instrument of choice for this challenging task. General unknown screening workflows do not use a target analyte list and compound detection is not based on any prior knowledge, including retention times and information on possible molecular and fragment ions. Therefore, acquired chromatograms are very rich in information and can easily contain thousands of ions from both any compounds

present in the sample as well as from the sample matrix itself. Thus, powerful software tools are needed to explore such data to identify the unexpected compound.

Water samples were collected both upstream and downstream of two WWTPs (Seymour and Fort Beaufort) and were directly injected on the AB SCIEX TripleTOF™5600 LC/MS/MS after being filtered. 15 sample points along the Kat River, ranging from a point as close to the source as possible to a point just before it joins the Great Fish River were used. The samples collected from the source were used as the control in each of the experiments, the assumption being the closer you get to the source, the less contaminated the water would be for the analysis of pesticides. Points were selected where the Kat River crosses the R67 or on farms where the river was accessible using farm roads. Samples were collected from October 2013 to November 2014.The Peak view software and Analyst software were used in the analysis of PPCPs. The XIC Manager allows you to manage large lists of compounds and perform automatic extracted ion chromatogram (XIC) calculations and review results operations. The results were displayed in the chromatogram pane and the XIC table (see results).

The results reported here in this thesis indicate that there is contamination in the Kat River water due to both pesticides and PPCPs. The results also indicate that the food products are also contaminated and hence both the Kat River agricultural produce and its water need to be closely monitored for both pesticide and PPCPs contaminants. Further studies to investigate the quantitative levels of pesticides and PPCPs in the Kat river water to determine if the concentration levels of the detected pesticides are below the reported Maximum Residues Limits will be explored in the future.

1 CHAPTER ONE

LITERATURE REVIEW

1.0 Introduction

Among the various compounds considered as emerging pollutants, Pesticides and Pharmaceuticals and Personal Care Products (PPCPs) are of particular concern, both because of the volume of these substances used and because of their activity as endocrine disruptors or as causative agents of bacterial resistance, as is the case of antibiotics. The detrimental environmental and health effects of pesticides on humans have been documented in the past decades. Prior to most of these studies, highly toxic pesticides were used in large quantities and in sensitive areas with great environmental and human exposure. For example, large-scale spraying of trees and plants in the 1960's was common. One pesticide used for this was DDT (dichlorodiphenyltrichloroethane) which has been shown to have significant health consequences.

In line with the debate on the effects of pesticides on the environment and human beings, the current research focuses on pesticide use and other contaminants (pharmaceuticals and personal care products) and possible pollution on Kat River in the Eastern Cape Province and their effects on crop plants cultivated on the Kat river banks and on the communities living near or on the river banks. Once pharmaceutical and pesticide compounds enter the environment, a number of question arise: are they transported along the watercourse and diluted to such levels whereby their presence becomes negligible, or do they adsorb onto solids and accumulate over time leading to increased concentrations, or are they degraded or transformed into various other chemicals in the presence of sunlight?

Carrying out this study is not only urgent, but also necessary because there are no known studies that have been conducted on Kat River with regards to the effects of pesticides on the environment and human life. The identification of these contaminants was accomplished by the use of the QuEchERS (Quick Easy Cheap Effective Rugged and Safe) extraction method for the extraction of pesticides from fruits and vegetables. This was followed by selective analysis using a liquid chromatography–mass spectrometry method for the identification of pesticides in fruits and vegetable samples. The direct injection method was used for the identification of pesticides, pharmaceuticals and personal care products in the water. Peak

intensity of identified compounds was used as an estimate of potential concentration levels of those particular contaminants, all other factors being constant.

Background

The following chapter presents a background information on pesticide, pharmaceutical and personal care products usage. Selective pesticide and pharmaceutical metabolic pathways are established in the chapter. The health and environmental impacts are stressed to demonstrate that pesticide and PPCPs contamination of water and food chain supplies is a current, significant problem for which continued data regarding non-point source pollution, downstream water healthy and food security should be gathered and recommendations established.

1.1 Pesticide water contamination

A pesticide is any substance, chemical, biological or otherwise, that is used for the purpose of preventing, destroying, or controlling pests. Pests may mean any species of plants or animals that interferes with the desired plants' growth and harms its production, processing, storage, transport, or marketing. Pesticides also include substances that are used before or after the desired plants are harvested to protect them during storage and transport (International Code of Conduct on the Distribution and Use of Pesticides, 2002). Ideally, an applied pesticide would target only the specific pest that is bothersome. This would be a narrow-spectrum pesticide. However, most pesticides are broad-spectrum and their effects cannot be limited to target individual pests. Beneficial organisms may be damaged by pesticides as well.

Historical development of pesticides

The development and use of pesticides was noted as a huge contributor to the green revolution. The application of pesticides as a way to improve crop yield and to aid in crop protection against a wide variety of insectivorous and herbaceous pests that would otherwise diminish the quantity and quality of food produce was a huge initiative. This initiative coincides with the "chemical age" which has transformed society since the 1950s. First generation pesticides refer to the pesticides commonly produced and used prior to the 1940's.These first generation pesticides were organic pesticides, naturally-occurring and typically withdrawn from plant compounds. When drawn from plants, pesticides are called botanicals. They do not persist in the environment and are easily degraded, but can be very toxic to aquatic life before degradation. Second generation pesticides refer to synthetic

pesticides produced after the 1940's, which are modified forms of botanicals which have more targeted effects on pests. Second generation pesticides are more poisonous than first generation pesticides and are more likely to persist in the environment. Their persistence depends on their class and type of pesticide. Currently, over 2,000 types of pesticide products are commercially available (Raven et al., 2008).The chronology of pesticide development is shown in Table 1-1.

Table 1-1: Chronology of pesticide development. Adapted from (Stephenson and Solomon., 1993)

Period	Example	Source	Characteristics
1800-1920s	Early organics, nitro-phenols, chlorophenols , creosote, naphthalene, petroleum oils	Organic chemistry, by-products of coal gas production, etc.	Often lack specificity and were toxic to user or non-target organisms
1945-1955	Chlorinated organics, DDT, chlorinated cyclodienes	Organic synthesis	Persistent, good selectivity, good agricultural properties, good public health performance, resistance, harmful ecological effects
1945-1970	Cholinesterase inhibitors, organophosphorus compounds, carbamates	Organic synthesis, good use of structure-activity relationships	Lower persistence, some user toxicity, some environmental problems
1970-1985	Synthetic pyrethroids , avermectins , juvenile hormone	Refinement of structure activity relationships, new	Some lack of selectivity, resistance, costs and

	mimics, biological pesticides	target systems	variable persistence
1985-	Genetically engineered organisms	Transfer of genes for biological pesticides to other organisms and into beneficial plants and animals. Genetic alteration of plants to resist non-target effects of pesticides	Possible problems with mutations and escapes, disruption of microbiological ecology, monopoly on products

Types of pesticides

There are many types of pesticides that target different types of pests: insecticides to kill insects, herbicides to kill harmful vegetation, rodenticides to kill rodents, fungicides to kill funguses, and so on. Pesticides may employ a number of different mechanisms to eliminate harmful pests. The types of pesticides used, classified by their treatment methods, include: chemical pesticides, biological pesticides, antimicrobials, and pest control devices. The major groups of chemical pesticides include organophosphates, carbamate pesticides, organochloride pesticides, and pyrethroid pesticides. They vary in the mechanism that targets and inactivates or inhibits pests (Bourgeois *et al.,* 2012).

1.1.1.1 Carbamates

Carbamate pesticides are insecticides that are derived from carbamic acid and function in a way similar to organophosphates, inhibiting the cholinesterase enzymes. They were first introduced in the 1950's and remain widely used because of their relatively low toxicity compared to other insecticides, particularly the organophosphates. Like the other types of insecticides, these can affect the human nervous system with routes similar to those that affect the target insects. Respiratory problems result from poisoning, but the inhibition of acetyl cholinesterase is reversible so short-duration exposure may not be extremely detrimental (Fishel, 2004). Two common carbamates are carbaryl and aldicarb.

1.1.1.2 Organochloride pesticides

Organochloride pesticides were used heavily in the 1940's-1960's but are not as widely used today since they have a high potential for chronic health effects and they persist in the environment for months or even years. These chlorinated hydrocarbons are broad-spectrum. They are primarily used as insecticides. They can include chlorinated ethane derivatives such as DDT, cyclodienes, and hexachlorocyclohexanes (Gold *et al.*, 2001). Some that remain in use today include alachlor, atrazine, lindane, and methoxychlor. The most famous type of organochloride insecticide is DDT (dichlorodiphenyltrichloroethane), perhaps one of the most well-known of all pesticides. The wide-spread toxic effects of DDT were studied by Rachel Carson and published in her 1962 book Silent Spring, which revealed the detrimental effects of pesticides on bird populations, particularly eagles and others at the top of the food chain, and the significant weakening of their eggs' shells. This book is sometimes credited for helping to truly launch the environmental movement and it was published prior to the formation of the US Environmental Protection Agency in 1970 (Raven *et al.*, 2008). DDT also has effects on the human immune system.

1.1.1.3 Organophosphates pesticides

Organophosphates (OPs) are insecticides that contain phosphorous and kill insects by targeting the enzymes that regulate the neurotransmitter acetylcholinesterase, disrupting brain function. Following the decreased usage of organochloride insecticides, organophosphates have become the most widely used today. They were originally developed during WWII. Some organophosphates are highly poisonous, comparable to poisons such as arsenic and cyanide. However, they degrade in the environment readily and do not have long-term environmental effects (United States Environmental Protection Agency, 2011). Because of this dichotomy; many organophosphates are used in large-scale agriculture settings but are not available on smaller scales because of their highly toxic properties. Some examples of organophosphates include glyphosate, dimethoate, and Malathion (Raven *et al.*, 2008).

1.1.1.4 Pyrethroids

Pyrethroids were synthesized to have the same effects as the naturally-occurring pesticide pyrethrum, extracted from the chrysanthemum flower, but be increasingly stable without persisting in the environment (United States Environmental Protection Agency, 2011). They are widely used. An example of a pyrethroid is cypermethrin. However, the effects of pyrethroids on the human immune system have not been extensively studied since they were developed relatively recently (Gold *et al.*, 2001).

Legislation on pesticide use

The South African government has passed many laws surrounding the use of pesticides. After a pesticide's application, laws also govern acceptable residue limits found on food and the allowable contaminant levels found in drinking water and surface water bodies. Internationally, the World Health Organisation and divisions of the United Nations work to maintain standards for pesticide use, in addition to foreign governments. The European Union also sets standards to regulate concentrations in water and on foods.

Domestic legislation

Some of the major laws governing pesticide use within South Africa are the Food, Drug, and Cosmetics Act (FDCA), the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the Food Quality Protection Act, the Clean Water Act, and the Safe Drinking Water Act. The Environmental Protection Agency (EPA) is empowered by these laws to monitor pesticide registration, use, and concentrations in foods and water supplies.

Food, drug, and cosmetics act (FDCA)

This act, originally passed in 1938, was amended in 1954 to allow for the establishment of standards for acceptable and unacceptable levels of pesticides found in food. This was the first means for regulating pesticide levels in foods. With a later amendment called the Delaney Clause added in 1958, it also specifies that no processed foods can contain any pesticides that have been shown to cause cancer in animals during laboratory tests. However, this clause did not cover raw foods such as vegetables, meats, or milk, and also was difficult to enforce since not a lot of data was available at the time to link specific pesticides to cancers (Raven *et al.*, 2008).

Federal insecticide, fungicide, and rodenticide act (FIFRA)

The FIFRA act passed in 1947 and amended in 1983 and 1988 requires the registration of all pesticides used in the US and sets standards for their distribution, sale, and use. This act was passed to help prevent the use of pesticides that were no longer usable and would instead cause damage to users or to the environment. When a pesticide is registered, the EPA investigates the type of pesticide, the area it is intended to be used and in what quantities, and the storage and disposal methods (Bourgeois *et al.*, 2012). The pesticide must meet the standards set by the FDCA in order to be granted registration (United States Environmental Protection Agency (EPA), 2011). However, some critics are dissatisfied that FIFRA does not

require pesticide manufacturers to disclose all the inert ingredients in the pesticides, only the active ingredients, when inert ingredients may pose health and environmental dangers as well, since some common inert ingredients include toxins such as benzene, lead, and formaldehyde (Raven *et al.,* 2008). For instance the surfactant Polyethylene Amine (POEA) in commercial formulations of glyphosate such Round Up has been shown to be much more acutely toxic to humans and wildlife than glyphosate alone. The lethal dose of POEA is less than a third of the lethal dose of glyphosate (Extension Toxicology Network, 2012).

Evaluation of carcinogenic potential

Carcinogenic potential is one important part of the hazard assessment that the EPA does. This involves laboratory testing using rats and mice. After research is conducted, the Cancer Assessment Review Committee assigns each active ingredient within the pesticide a cancer classification, which the EPA then uses to determine regulations surrounding the pesticide's use (Bourgeois *et al.,* 2012).

Based on the research conducted, pesticides can be assigned one of five different levels of carcinogenicity. The different levels of carcinogenicity are shown in figure 1-2.

Table 1-2: Levels of Carcinogenicity (Adapted from Fishel, 2004)

Level	Description
1	Carcinogenic to humans – though studies are based on animals, similar mechanisms observed between animals and humans can suggest conclusions that some compounds are likely to be carcinogenic in humans. The only pesticides of this group that are registered for legal use are arsenicals, but the use of these, typically for wood treatment, is strictly monitored and has been reduced significantly as of 2003.
2	Likely to be carcinogenic to humans – this classification results from data that demonstrates carcinogenic potential. An example from this group is imazalil, a fungicide used in citrus agriculture.
3	Suggested evidence of carcinogenicity, but not sufficient to asses human carcinogenic potential – further studies are required to determine the true human

	carcinogenic potential in this case. Pyrethrins fall into this category.
4	Data are inadequate for an assessment of human carcinogenic potential – there is a lack of sufficient data, or different studies may contradict one another. Further studies are required. Pyraclostrobin, used for fruits and vegetables, is an example from this category.
5	Not likely to be carcinogenic to humans – studies have revealed that the pesticide does not pose a threat to humans. This may be deduced from studies of human exposure or from animal studies that are shown to be relevant to humans. An example would be glyphosate.

Food quality protection act

Passed in 1996, the Food Quality Protection Act amended both the FDCA and FIFRA to set pesticide residue limits for all foods—raw or processed. It also refined the pesticide limits to include all health risks rather than simply cancer, and to take into consideration the higher risks that children and infants face. This act also sharply reduces the amount of time between when a pesticide is banned to the time it must be removed completely from use (from 10 years to 14 months) (Raven *et al.,* 2008).

Clean water act

The Clean Water Act (CWA) originally passed in 1948 and expanded in 1972 with amendments in 1977, is the primary federal law monitoring water quality which sets the structure for regulating concentrations of pollutants in surface water supplies. Ground water is not described in this law. It protects "navigable" water bodies by limiting point source discharges that manufacturers and other facilities may make into surface water bodies with the goal of making them safe for fishing and swimming (Davis and Masten, 2004). Non-point sources are more difficult to monitor and regulate, and the approach for this involves education, technical assistance to manufacturers, and similar approaches. Water quality standards specify water quality standards (WQS) for allowable pollutant levels that water bodies must meet, involving total maximum daily loads (TMDLs) (United States Environmental Protection Agency (EPA), 2011). Human life criteria consider pollutants effects upon humans and the environment (Davis and Masten, 2004).

Safe drinking water act

This act was first passed in 1974 and amended in 1986 and 1996. It regulates drinking water quality in public water supply systems as well as their sources. The EPA sets primary and secondary drinking water standards that water treatment facilities must comply with before discharge to public water distribution systems. These standards involve treatment processes that must be included, as well as permissible contaminant levels in the plant's effluent. States may also set their own drinking water standards as long as they are at least as stringent as the national standards (United States Environmental Protection Agency (EPA), 2011).

Under this portion of the law, both drinking water health regulations and advisories are made. The National Primary Drinking Water Regulations specify maximum contaminant levels (MCLs) of a contaminant that is the highest permissible and safe concentration in water discharged to public water systems. These contaminants include microorganisms, disinfectants and by-products, inorganic chemicals, organic chemicals, and radionuclides. Many pesticides have specified MCLs, including alachlor (0.002 mg/L), atrazine (0.003 mg/L) and glyphosate (0.7 mg/L) (United States Environmental Protection Agency (EPA), 2011).

International legislation

Internationally, the International Code of Conduct on the Distribution and Use of Pesticides was passed in 1985 by the United Nations' Food and Agriculture Organization (FAO) and revised many times since then, the latest in 2002, to set voluntary standards for the use of pesticides. Though countries are not obligated to abide by these standards, they help raise awareness of the potential consequences associated with use of pesticides and serve as a reference that is considered the "globally accepted standard for pesticide management" (International Code of Conduct on the Distribution and Use of Pesticides, 2002). National legislatures maintain the standards that each country must abide by, but in some cases, particularly in developing countries, monitoring compliance with laws is difficult.

European Union legislature

More formally, the European Union (EU) has detailed legislation surrounding the use of pesticides in member nations. In doing this, the EU has separated its legislation into two main divisions: the classification and usage of pesticides, and the official maximum residue level for each compound. These two divisions work together to set a standard for pesticide restriction, in order to keep the general public safe (Bourgeois *et al.*, 2012).

Classification and usage of pesticides

The first initiative in which the European Union started to standardise the restriction and legislation of pesticides occurred in 1993, when Directive 91/414 was passed by the EU. This directive stated that over the next 14 years, a council was to be created to review all pesticides, their uses and the products in which they were found. The review would establish whether the pesticides and products were harmful to the community and would either allow continued production of the pesticide or ban the product and/or pesticide. This stemmed from concern regarding the effects of pesticides on the food market both within and outside the European Union (SCI. Proposals to restrict the use of pesticides in the European Union, 2011).

The classification of pesticides, would be based on information from manufacturers, regarding pesticide efficiency, main purpose, and potential harm to humans and the environment. The classification would simply list the chemicals followed by this information. The EU would then make a decision to either ban or allow the continued production of this pesticide. This process, otherwise known as risk assessment, took into account the possible damage the pesticide could present should there be a contamination in the local water system. Risks to humans as well as the possible risks to the environment and wildlife were of paramount importance. In December of 2008, the review process was extended until 2010 and then later until 2012. Prior to this directive, the legislature on pesticide restriction was dealt with on the national level only (SCI. Proposals to restrict the use of pesticides in the European Union, 2011)

The next directive, Directive 2009/128/EC, was passed in November 2009 and states that each national community should develop and/or adopt a National Action Plan. This plan was to be used at national level to reduce the risk to human and environmental life when dealing with pesticide usage. This Directive focuses on pesticide concentration in food products. The plan was passed with the hope that it would help to advocate the research and development of new techniques and delivery methods (SCI. Proposals to restrict the use of pesticides in the European Union, 2011).

In May 2011, the Plant Protection Products Regulation Act established a list of approved chemicals and products for use in the European Union. This list shows the accepted purity, date of approval, as well as the expiration of approval. Once a pesticide's approval expires, the review committee re-evaluates the chemical and may either continue to approve the usage

or ban the pesticide. Coinciding with Directive 91/414, a list was also established which lists banned substances in the European Union (Food & Fairness Briefing No. 1, 2008).

Maximum residue Level

The second division of the European Union's pesticide legislation involves the investigation of each pesticide in order to establish the maximum residue level. This maximum residue level is the maximum concentration that is found in local crops. The European Union defines these levels as the "highest possible level of a pesticide residue that is legally authorised in food and feed." In September 2008, the EU passed Regulation 396/2005. This regulation set a standard for maximum residue levels in all EU governed states. This regulation was to ensure that a product would not be legal in one country and yet above the maximum residue level in another community (European Commission: Health and Consumers, 2012).

Health impact of pesticides

In addition to the desired effects on targeted pests, pesticides can also have detrimental effects on human health. Acute effects occurring within minutes or hours after a single exposure, as well as chronic effects spanning multiple exposures and weeks to years can be sustained. Chronic effects are much more dangerous than acute effects because the effects are wide spread and difficult to monitor. Chronic effects normally show up following continuous individual exposure to pesticides and there are various mechanisms through which individuals can get in contact with pesticide resulting in different symptoms (Bourgeois et al., 2012).On the other hand acute toxicity effects are much less difficult to monitor. Chronic toxicity is depended on individual's degree of contact with the pesticides. Personal health and other factors such as individual's genetics also contribute the extent to which how chronic toxicity can be. In order to determine the extent of toxicity following either acute or chronic exposure to pesticides, various tests can be conducted by simply subjecting test animals to pesticides at various concentration levels. Long term effects and short effects can be determined in this way as this can simulate human effects (Bourgeois et al., 2012).

Toxicity of pesticides

Dermal contact is responsible for the majority (approximately 90%) of pesticide poisonings, typically during pesticide application, handling, or other routine uses (Nesheim *et al.*, 2009). Ingestion and inhalation are the other means. The seriousness of dermal exposure and the degree of the effects depend on the rate of absorption of the substance through the skin, the size of the area of skin exposure, the length of contact time, the number and concentration of

the substances that contacted the skin, and of course, the level of toxicity of the pesticide(s). Pesticides that volatilise can be inhaled from the atmosphere. Eye irritation can also result from direct contact with pesticides.

Acute toxicity levels are measured by the half lethal dose, or LD50: the dosage at which 50% of animals exposed to the substance were killed. The lower the LD50 is for a particular pesticide, the greater the toxicity. This acute toxicity level determines the type of labelling required for pesticide containers, to help warn users of their dangers. Table 1-3 shows the acute toxicity level standards. Highly toxic pesticides must have the words "danger" and "poison" displayed on them, as well as the universally understood skull and crossbones picture. Only a few drops of highly toxic pesticides could be fatal for a 150lb/person. Moderately toxic pesticides have "warning" labels and either slightly toxic or relatively non-toxic pesticides read "caution." Even pesticides classified as relatively non-toxic can still be hazardous if proper care is not taken to use them as directed and avoid excessive exposure (Hock *et al.,* 2006).

Table 1-3: Acute Toxicity Measures and Warnings (Adapted from (Nesheim *et al.,* 2009))

			LC_{50}	LD_{50}	LD_{50}
Categories	Signal word	Oral lethal dose	Oral mg/kg	Oral mg/kg	Dermal mg/kg
1.Highly toxic	DANGER, POISON (skull & crossbones)	a few drops to a teaspoonful	0 to 0.2	0-50	0-200
2.Moderatley toxic	WARNING	over a teaspoonful to one ounce	0.2 to 2.0	50-500	200-2000
3.Slightly toxic	CAUTION	over one ounce to one pint	2.0 to 20	500-5000	2000-20000
Relatively	CAUTION (or	over one pint to one	20 +	5000+	20000+

non-toxic	no signal word)	pound			
Probable for a 150 lb.-person.					

To avoid excessive exposure and help protect against pesticide-induced health risks when handling them, manufacturers recommend some levels of minimum person protective equipment (PPE) which typically includes long pants and sleeves, shoes, gloves, and possibly safety glasses and a face mask for more toxic pesticides.

Table 1-4 lists some common herbicides in use in the US, their active ingredients, and the acute oral and dermal LD50 values. REI, or restricted-entry interval, is the amount of time necessary between the application of the pesticide to crops and the safe re-entry of humans into the area is permitted and is also listed. This is a partial excerpt taken from a list of 77 pesticides for which LD50 values were reported (Hock *et al.,* 2006).

Table 1-4: LD_{50} Concentrations and Restricted-Entry Intervals for Selected Herbicides (Adapted from (Hock *et al.*, 2006))

		LD_{50} Values(mg/kg)		REI
Active Ingredient, Trade Name	Use Category	Oral	Dermal	(Hours)
Acetochlor, Degree	R	2,148	4,166	12
Acifluorfen, Blazer	G	2,025	>2,000	48
Alachlor, Lasso, Partner	R-12	930-1,350	13,300	12
Ametryn, Evik	G	1,950	-	12
Asulam, Asulox	G	>5,000	>2,000	12
Atrazine, AAtrex	R	1,869	>3,100	12
Bensulide, Prefar	G	271-1,470	-	12

Bentazon, Basagran	G	2,063	>6,050	12
Bromoxynil, Brominal, Buctril	G	260	>2,000	12
Butylate, Sutan +	G	4,500	>4,640	2
Carfentrazone-ethyl, Aim	G	5,143	>4,000	12

Pesticides and human effects

Organisms that come into contact with pesticides typically suffer negative health effects, either acute or chronic, that have the potential to be very severe. As desired, pesticides are acutely toxic to pests and work to inactivate them; thus this highly toxic nature can be expected to have similarly detrimental effects on both animals and humans, varying with the length of the exposure and the dose of the pesticide. These health effects can result from direct contact with pesticides, but also indirect contact when an individual drinks contaminated water or consumes contaminated foods (Bourgeois *et al.,* 2012).

Bioaccumulation and biomagnification of pesticides

Persistent pesticides that do not readily degrade can be stored within plants and later consumed by and transferred to animals and humans. Since many of these pesticides can be fat-soluble, they accumulate within fatty tissue of animals and humans. As subsequent animals consume the previous animals, the pesticide components remain within the system. High concentrations of the pesticide are stored. Moving up the food chain, organisms closer to the top have higher concentrations of these pesticides within their tissues. This phenomenon of greater concentrations of pesticides with successively higher levels on the food chain is called bio-magnification. Population groups that consume large amounts of fish and wildlife may be at increased risks of health consequences due to bioaccumulation of toxic pesticide compound (Raven *et al.,* 2008).

A group within the EPA focuses on persistent, bio accumulative, and toxic (PBT) pesticides' effects and monitoring, since these pose serious health issues that remain for years (United States Environmental Protection Agency, 2012). These Level 1 PBT pesticides are aldrin, dieldrin, chlordane, DDT, mirex, and toxaphene; all highly chlorinated and degrade very slowly (University of California – Berkeley, 2010).

Short-term effects of pesticides

Humans that are exposed to large doses of pesticides even for short periods of time can experience serious health effects. These may range from nausea and vomiting, and even more serious consequences, such as death, depending on the type of pesticide and the dosage. The individuals' prior health also plays a significant factor, but high doses of pesticides can be fatal. Organophosphates, in particular, tend to have extreme acute effects. According to the World Health Organisation (2004), 300,000 people die annually from pesticide poisoning worldwide, while a total of four million suffer other health effects from some form of poisoning. This is often due to improper handling of pesticides and not necessarily only due to the transport and transfer of pesticides in the environment (Raven *et al.*, 2008). Neurological disruptions including headaches, dizziness, nausea, vomiting, confusion, tremors, and convulsions are possible, as well as eye, nose, mouth, and throat irritation (Raven et al., 2008).

Long-term effects of pesticides

Chronic exposure to pesticides can have a wide range of health effects. Pesticides have been linked to many different types of cancers including lymphoma, leukaemia, and brain, lung, and testicular cancer. Breast cancer may be linked to pesticides since they tend to bioaccumulate within breast tissue, but further research must be conducted to definitively prove the correlation. Long-term exposure is also responsible for causing sterility both in humans and in other animals. Miscarriages also have been linked to pesticide exposure. Another disease that may be related to contact with pesticides is Parkinson's disease. With each of these long-term illnesses, identifying a definitive correlation between exposure to a specific pesticide and the illness can be difficult since large sample populations may not be available, and many other factors could play into the individual's illness (Raven *et al.*, 2008).

Level 1 pesticides are all classified as probably carcinogens. Some have been linked to central nervous system damage and neurological system disruption, damage to the liver, kidney, thyroid, reproductive system, and digestive system. Some may cause neurological disorders in children whose mothers are exposed during nursing or before giving birth. Many are suspected endocrine disruptors (United States Environmental Protection Agency, 2011).

Pesticides as endocrine disruptors

Many different types of pesticides have been shown to affect the endocrine hormones, such as oestrogen and testosterone, as well as alter the reproductive systems or organs of animals.

These endocrine disruptors can affect many different types of organisms, such as fish, amphibians, birds, reptiles, laboratory rats, and even humans. For example, male frogs that were exposed to atrazine were found to turn into females from lack of testosterone. 75% of male frogs were emasculated and 10% were turned into females. When these atrazine-induced females then mated and produced offspring, all offspring were male, skewing the sex-ratio of frogs in that population (Bourgeois et al., 2012).

Research into possible endocrine disruption in humans, has yet to determine the long term potential effects of these pesticides. It could take decades to observe the long-term effects and understand the root causes. Table 1-5 lists some commonly used pesticides that have been shown in laboratory research with animals to be endocrine disruptors, and whether or not they are still used within the US (Raven *et al.,* 2008).

Table 1-5: Known Endocrine Disruptors Used in the US (Adapted from (Raven *et al.*, 2008))

Pesticide	General information
DDT (dichlorodiphenyltrichloroethane)	Insecticide; banned in US in 1972
Methoxychlor	Insecticide; still used
Kepone	Insecticide; banned in US in 1977
Atrazine	Herbicide; still used
Endosulfan	Insecticide; still used
Chlordane	Insecticide; banned in US in 1988

Pesticides and health effects on young children

Since infants and children have much less developed immune systems and much greater cell division rates as they grow, they are more susceptible to the dangers posed by pesticides. Their biochemical and physiological functions are largely immature compared to those of grown adults, in addition to smaller proportions of organs, muscles, bones, and brains.

Therefore, toxicity of pesticides can be increased due to different absorption, transport, and metabolism rates. In the beginning months of pregnancy, toxic compounds can have permanent damage to foetuses, but many dangers still exist after birth. During the continual development of the central nervous system, pesticides that have neurotoxic effects (such as organophosphates, organ chlorides, and carbamates) can be particularly toxic even in low doses (Nesheim *et al.*, 2009).

In Garry's 2004 review paper considering previous studies of the health impacts upon children, he notes that some studies have shown altered sex ratios (more females than males), increased occurrence of miscarriages, and "significantly increased numbers of birth defects" (Garry, 2004) compared to children either living in non-agricultural communities or not exposed to pesticides in direct studies. In particular, he points to one study from the Minnesota Red River Valley farm community, where increased birth defects were observed in families where fumigant phosphine and herbicide glyphosate were used. Since the sample population was only around 1500 children, he calls for further studies to support this. From the same review paper, he concludes that childhood cancers are "weakly but consistently associated with pesticide use and in particular paternal pesticide use." (Garry, 2004) Neurodevelopmental studies indicate lower short-term memory and deficient motor skills in children with multiple pesticide exposures. Linkage to attention deficient disorders or hyperactivity has been theorised but has not been supported by studies yet (Garry, 2004).

Overall illnesses caused by pesticides.

Table 1-6 lists the relative number of illness that each type of pesticide was responsible for in the US in 1996. This includes minor, moderate, major, and fatal illnesses and these are only illnesses which were reported to the poison control centre, so it cannot be considered completely comprehensive. According to the American Association of Poison Control Centres, many of these illnesses could have been avoided with proper treatment after the exposure, such as dilution of the pesticide with sufficient water. However, some of the more toxic pesticides required medical attention to remediate the effects. This list includes organophosphates, pyrethrins/pyrethroids, hypochlorite disinfectants, carbamantes, organochlorides, phenoxy herbicides, and anticoagulant rodenticides (Nesheim *et al.*, 2009).

Table 1-6: Pesticides Most Often Implicated in Symptomatic Illnesses, 1996 (Adapted from (Nesheim *et al.*, 2009)).

Rank	Pesticide or pesticide class	Child <6 years	Adults and those 6 – 19 years	Total*
1	Organophosphates	700	3,274	4,002
2	Pyrethrins and pyrethroids**	1,100	2,850	3,950
3	Pine oil disinfectants	1,136	903	2,246
4	Hypochlorite disinfectants	808	1,291	2,109
5	Insect repellents	1,081	997	2,086
6	Phenol disinfectants	630	405	1,040
7	Carbamate insecticides	202	817	1,030
8	Organochloride insecticides	229	454	685
9	Phenoxy herbicides	63	387	453
10	Anticoagulant rodenticides	176	33	209
	All other pesticides	954	3,604	4,623
	Total all pesticides and disinfectants	7,279	15,015	22,433

*Totals include a small number of cases with unknown age.

**Rough estimate: includes some veterinary products not classified by chemical type.

Source: American Association of Poison Control Centres, Toxic Exposure Surveillance System, 1996 data.

Fate of pesticides in the environment

When pesticides are introduced into the environment, they may either be degraded over time or they can remain in the environment. If they persist, they may be adsorbed into the soil or transported through water flow. The various fates of pesticides present in the environment

(Fishel, 2005) are shown in Figure 1-1. The accumulation or transport of pesticides in the soil determines possible groundwater leaching which can directly affect human toxicity issues. Individuals who come in contact with treated soil are also susceptible to health issues, such as children that are playing on lawns or in fields that have been treated.

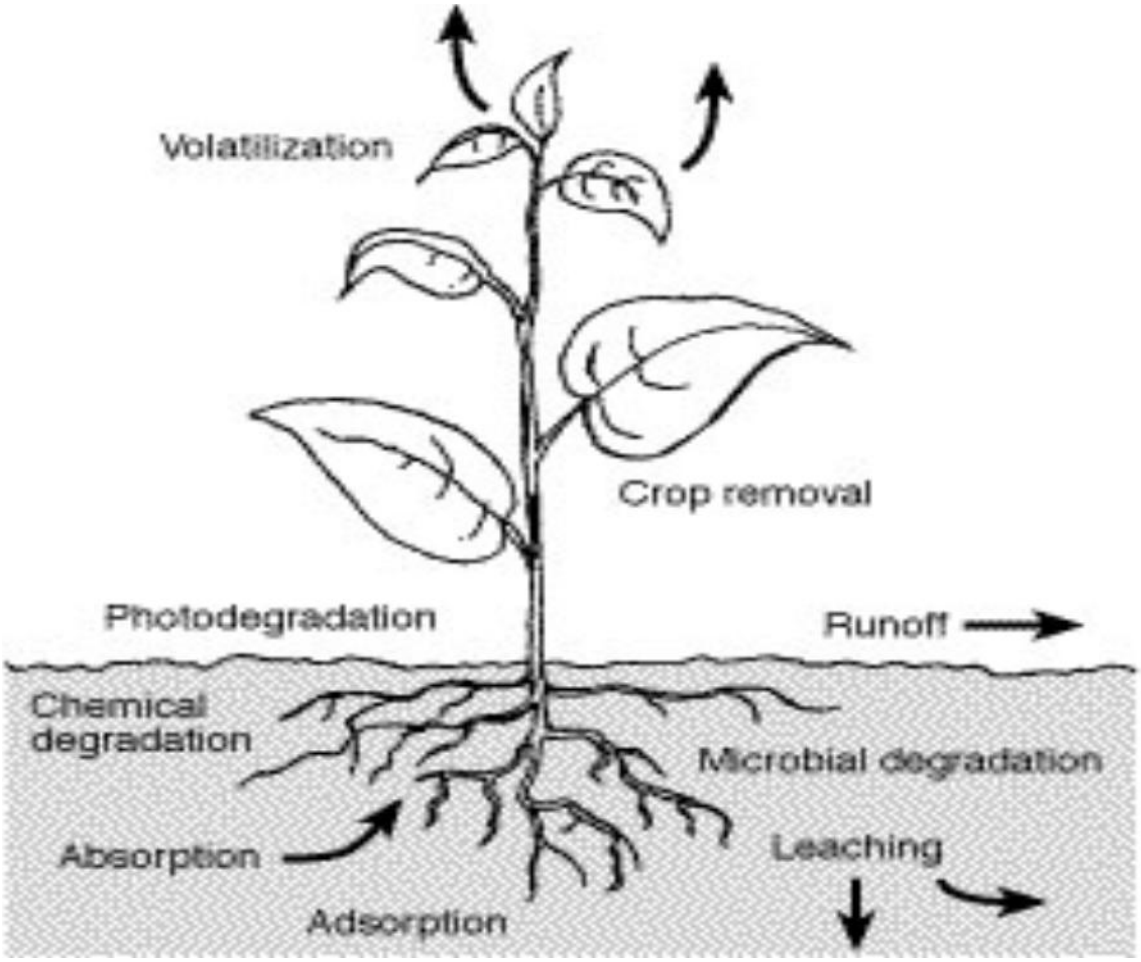


Figure 1-1: Pesticides Fate in the Environment (Adapted from (Fishel, 2005))

Possible sources and entry pathways of pesticides into the environment.

There are various ways through which pesticides can end up in the environment. The two main categories are diffuse or nonpoint source pollution and point source pollution. Pollution of waste water, be it non-point or point source, can be though empty pesticides containers being disposed directly into water bodies or washing of equipment after their application with surface water. Over the years, many attempts have been on controlling point source pollution. However, not as much effort has been put in controlling diffuse source pollution on natural

water bodies. Figure 1-2 is a summary of possible entry pathways of pesticides into the environment.

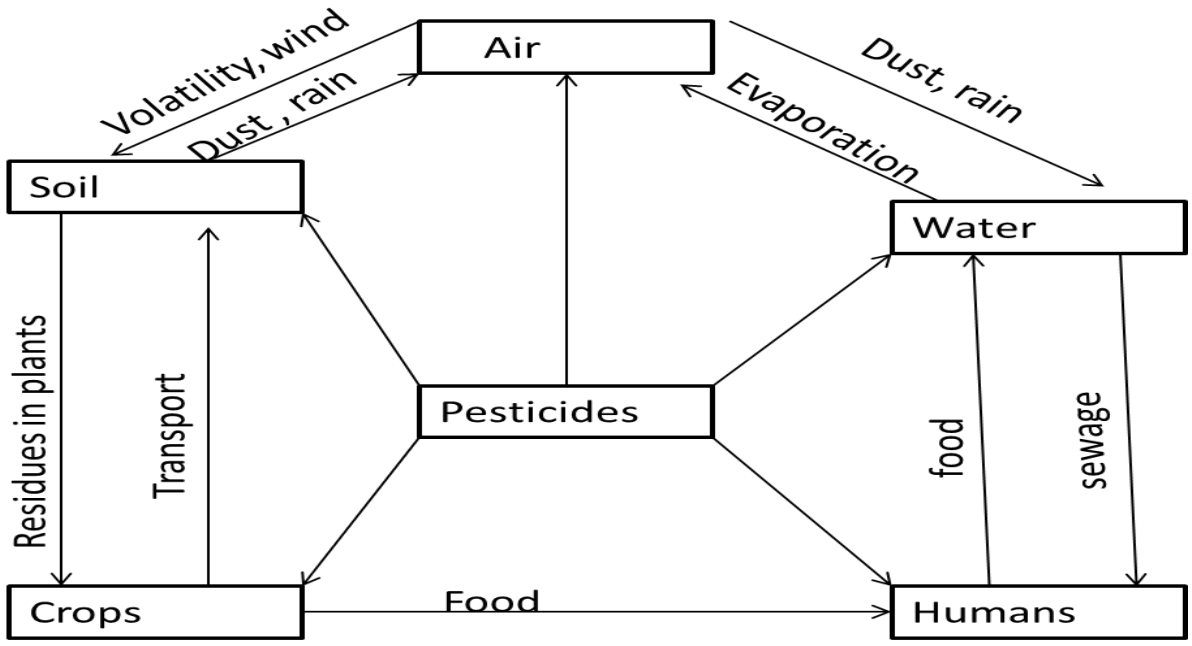


Figure 1-2: Possible sources of pesticides and entry pathways in to the environment (adapted from (Bourgeois et al., 2012))

Non-point source pollution defined

Diffusion or non-point source pollution is the pollution whose origins are not known. It emanates from various human activities and the pollutants thereof have no noticeable entry points into water bodies or rivers. This makes diffuse source pollution very difficult to control because of its multiplicity of origins. There is no a well-defined source over which you can put control measures on (Bourgeois et al., 2012).

Emerging problem

Irrespective of source, diffusion source pollutants end up in the receiving water bodies such as wetlands, rivers and lakes and, finally, to oceans mainly through leaching and direct runoff or melting snow. Therefore, downstream activities are affected posing a huge threat to aquatic life, water health and food security ranging from simple nuisance substances to severe ecological impacts. The range and relative complexity of agricultural diffuse source pollution are illustrated in Figure 1-3

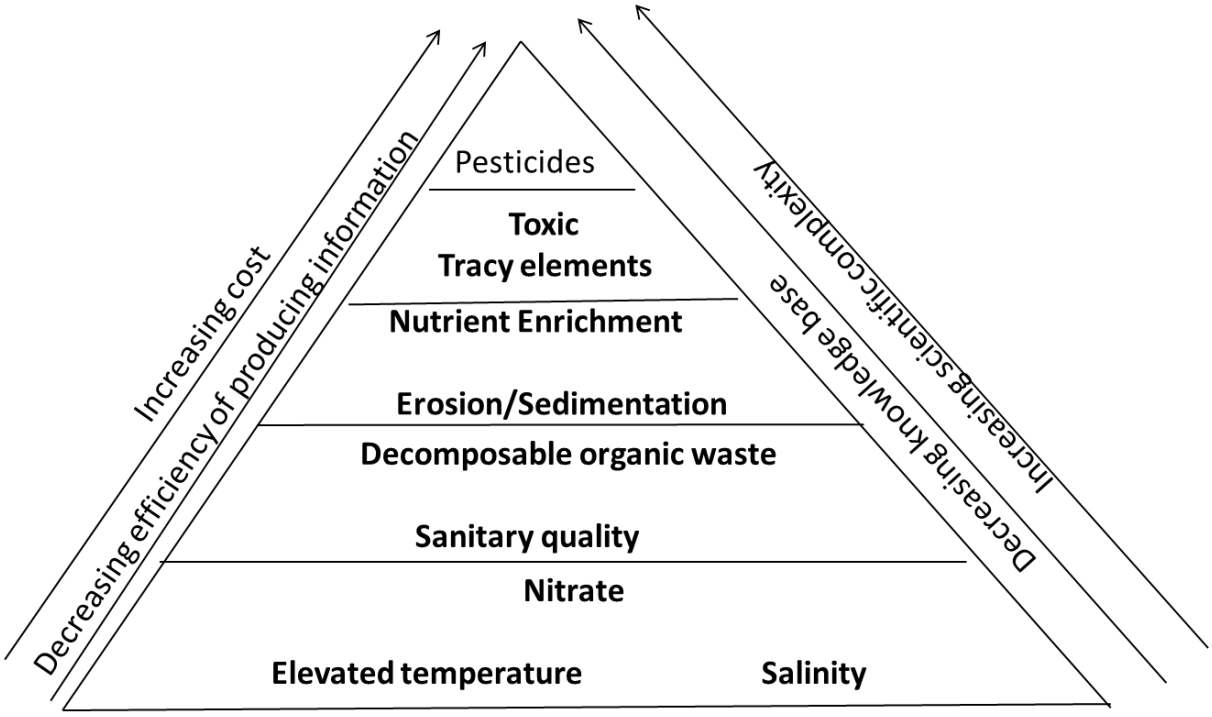


Figure 1-3: Hierarchical complexity of agriculturally-related water quality problems

Adapted from (Rickert, 1993)

Factors affecting pesticide toxicity

The ecological impacts of pesticides in water are determined by the following criteria presented in Table 1-7

Table 1-7: Factors that determines the ecological impacts of pesticides in water (Adapted from (A. Masiaa, et al 2013)).

Toxicity:	Mammalian and non-mammalian toxicity usually expressed as LD50 ("Lethal Dose": concentration of the pesticide which will kill half the test organisms over a specified test period). The lower the LD50, the greater the toxicity; values of 0-10 are extremely toxic (OMAF, 1991).

	Drinking water and food guidelines are determined using a risk-based assessment. Generally, Risk = Exposure (amount and/or duration) × Toxicity.
	Toxic response (effect) can be acute (death) or chronic (an effect that does not cause death over the test period but which causes observable effects in the test organism such as cancers and tumours, reproductive failure, growth inhibition, teratogenic effects, etc.).
Persistence:	Measured as half-life (time required for the ambient concentration to decrease by 50%). Persistence is determined by biotic and abiotic degradation processes. Biotic processes are biodegradation and metabolism; abiotic processes are mainly hydrolysis, photolysis, and oxidation (Calamari and Barg, 1993). Modern pesticides tend to have short half-lives that reflect the period over which the pest needs to be controlled.
Degradates:	The degradation process may lead to formation of "degradates" which may have greater, equal or lesser toxicity than the parent compound. As an example, DDT degrades to DDD and DDE.
Fate (environmental:	The environmental fate (behaviour) of a pesticide is affected by the natural affinity of the chemical for one of four environmental compartments (Calamari and Barg, 2008): solid matter (mineral matter and particulate

	organic carbon), liquid (solubility in surface and soil water), gaseous form (volatilization), and biota. This behaviour is often referred to as "partitioning" and involves, respectively, the determination of: the soil sorption coefficient (KOC); solubility; Henry's Constant (H); and the n-octanol/water partition coefficient (KOW). These parameters are well known for pesticides and are used to predict the environmental fate of the pesticide.

Water contamination includes but is not limited to pesticides. Pharmaceuticals, illicit drugs, personal care products and other substances coming from human activity provide a cocktail of organic contaminants that may result in multiple substances acting in “an additive, synergistic or antagonistic manner” that may render impacts relatively difficult to discern (A. Masiaa, et al 2013).

1.2 Pharmaceuticals and Personal Care Products (PPCPs) as emerging water contaminants

PPCPs are group of chemicals which are either used for personal health use such as food supplements, nutritional supplements, sports nutrition or used for personal care such as cosmetics and fragrances just to name a few. The uses of these chemicals have caught the eyes of researchers and scientists at global level as they are suspected to pose a huge threat to the environment. This has been worsened by the increased daily uptake of PPCPs. Some of the negative impacts which PPCPs are suspected to cause include the spread of antimicrobial resistance and in some instances elevated reproductive impairment. PPCPs are also suspected to have long term effects such as causing cancer if not monitored properly. Although by nature they are designed to serve for the manufacturing purpose and to degrade in aqueous environment, PPCPs are still detected in aqueous environment including surface water

bodies. Also because of their continuous influx into the environment, they don't need to be persistent for them to cause a negative impact to the environment.

PPCPs are known to be persistent in the environment. If they do degrade in aqueous environments, they do so slowly. They are also known to be bioactive and do not completely metabolize (Debska et al., 2004; Hernando et al., 2006; Ku¨mmerer, 2008).Not until recently, when scientists began to pay attention to the potential environment threat PPCPs might pose. Much focus was put on pesticides and other contaminants. The interest in PPCPs began early in the 1970s but again the main focus to screen for PPCPs as potential environmental threat both to aquatic life and human beings began recently (Daughton, 2002; Debska et al., 2004; Fatta et al., 2007; Heberer, 2002; Hernando et al., 2006; Ku¨ mmerer, 2009; Stan and Heberer, 1997; Zuccato et al., 2006).

It has come to many scientists and researchers knowledge that there is a huge influx of PPCPs into the environment due to human activity. Many people have access to over the counter medications such as antibiotics and steroids and the unwanted medication also end being flushed to sewers. Pharmaceutical industries also known to cause more damage as their wastes are disposed in waste water, Agribusiness also do the same. Hospitals are also continually disposing their residues and wastes, not even to mention the sky rocket use of veterinary and illicit drugs (Bartelt-Hunt et al., 2009; Escher et al., 2011; Larsson et al., 2007; Ternes, 1998).

Once PPCPs are disposed into the aqueous environment, one expects them to dissolve or degrade into by-products. Eventually they will enter the aquatic systems through various ways such as sewage or treated sludge and end up in the soil in many ways including irrigation with river water in which effluent has been discharged (Cunningham, 2008; Nikolaou et al., 2007).According to recent studies, it has been reported that current WWTPs partially remove PPCPS and because of that, the effluent receiving water bodies have been seen to be contaminated. PPCPS have even been detected in the drinking water (Benotti and Brownawell, 2007; Debska et al., 2004; Joss et al., 2008).Once released in the environment or receiving water bodies ,PPCPs remain persistent and unchanged in the environment in which case they remain bioactive even at low concentration level. Unknown biochemical reactions occur when PPCPs combine in the aqueous media and above all, they can also accumulate in the food chain. This can pose a serious health impact to the general public and also to aquatic animals. (Escher *et al.,* 2011; Hernando *et al.,* 2006; Ku¨ mmerer, 2008).

Recent literature indicates that the flux of pharmaceuticals from municipal sewage treatment plants (STP) is a considerable source of chemical pollution in surface, ground, marine, and even tap and bottled waters (Chang *et al.,* 2007; Heberer, 2002; Khan and Ongerth, 2002; Kolpin *et al.,* 2002; Rosal *et al.,* 2010; Ternes, 1998). For instance, an investigation conducted by the U.S. Geological Survey in 1999 to check the occurrence of PPCPs (e.g. sterols, hormones, pharmaceuticals, antibiotics) in surface and ground water has confirmed the presence of at least one PPCP at low levels in more than two thirds of the samples, with steroids, nonprescription drugs, and pesticides being the most frequently detected compounds (Kolpin *et al.,* 2002). Although the concentrations of individual pharmaceuticals reported from waste water are low and may not cause any harm to the human health, chronic exposure to a mixture of such compounds may disturb the balance in the human body and enhance a dangerous resistance to antibiotics and consequently pose a threat to the health of living organisms; a task that many scientists are currently investigating (Escher *et al.,* 2011; Hernando *et al.,* 2006; Santos *et al.,* 2007; Schriks *et al.,* 2010).

Sources of pharmaceuticals in the environment

The detection of a multitude of pharmaceutical compounds in the environment led to the question: how such speciality compounds, specifically designed for use in human and veterinary medical practice could end up in ground and surface waters? When compared to other aquatic pollutants such as pesticide residues, the entry of pharmaceuticals into the environment depends on a number of integral factors (Daughton, 2001). These factors include the overall pharmaceutical consumption rate, the pharmacological fate of the drug within the body, the behaviour of the drug during the wastewater treatment process and the ability of the receiving water to provide adequate dilution (Hirai et al., 1997; Akay and Ozkan, 2002, and (Dubois et al., 2011)). Information concerning each factor is important when attempting to predict which pharmaceuticals may be present in the environment. Possible sources of entry of pharmaceutical compounds are presented in Figure 1-4

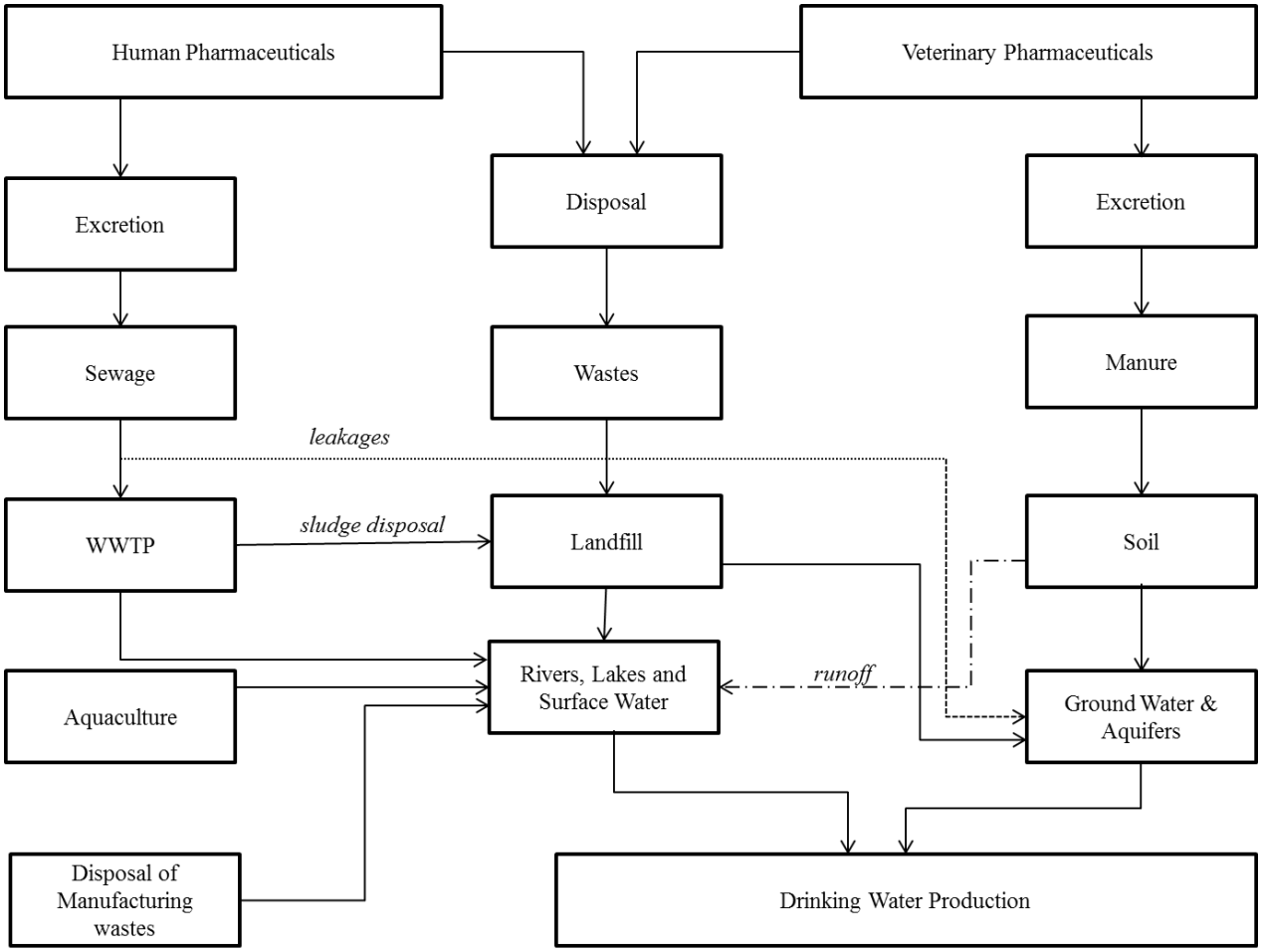


Figure 1-4: Sources of Pharmaceuticals (Adapted from (Dubois et al., 2011)).

The role of drug metabolism:

Pharmacokinetics is the branch of pharmacology that describes the processes affecting the absorption, distribution, metabolism and elimination of pharmaceutically active compounds in the body (Kourosh *et al.*, 1999). From the perspective of PPCPs as environmental pollutants, the most important pharmacokinetic process is drug metabolism as it provides information as to whether a drug will be metabolised within the body or be excreted in an unchanged form or if metabolism does occur, the proportions that will be excreted as parent molecule and metabolites or the types of metabolites that might be expected, i.e. which metabolic pathway dominates.

An important aspect of drug design is that of drug delivery, i.e. ensuring that the compound arrives at the desired site in the desired form to evoke its pharmacological effect. In order to cross cell membranes, pharmaceuticals must possess sufficient lipophilicity and consequently

the primary function of drug metabolism is to transform these lipophilic compounds into more polar metabolites that are suitable for elimination and excretion primarily through the kidneys via the urine (Rang *et al.,* 1995). Metabolism is an enzymatic process and involves transformation of the compound via Phase I and Phase II reactions. Phase I processes involve the functionalisation of the parent molecule in preparation for Phase II processes to occur, and normally consists of the addition or activation of a reactive functional group on the parent molecule. Typical Phase I processes include oxidation, reduction, hydrolysis, hydration or de-alkylation (Gibson and Skett, 1994). These reactions are predominantly governed by cytochrome P450 microsomal oxidase enzymes located in the endoplasmic reticulum of cells and require the presence of cofactors such as nicotinamide adenine dinucleotide phosphate (NADPH). Phase II processes involve the conjugation of an extremely polar moiety to the Phase I product, leading to a hydrophilic drug conjugate that is readily removed by Glomerular filtration in the kidney. Typical conjugates include sugars or glucuronides, sulphate, amino acids, glutathione or acetyl groups (Gibson and Skett, 1994). A diverse group of enzymes regulate Phase II reactions individual to the conjugate, e.g. sulphotransferases, glucuronyltransferases etc. each requiring its own individual cofactor such as 3'phosphoadenosine-5'-phosphosulphate (PAPS), or uridine diphosphate (UDP), respectively (Gibson and Skett, 1994). An example of Phase I & II processes is depicted in Figure 1-5 (Rang *et al.,* 1995).

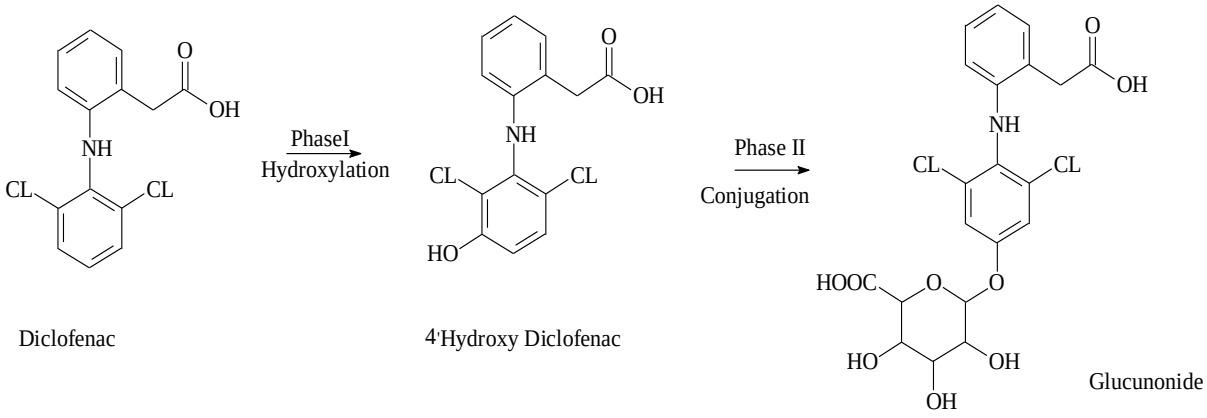


Figure 1-5: The metabolism of non-steroidal non-inflammatory drug Diclofenac (adapted from (Rang et al., 1995)).

As demonstrated by Figure 1-5 most pharmaceuticals are metabolised into a certain array of metabolites. However, the process becomes considerably more complicated as the enzymes involved in the metabolic reactions may be induced or inhibited by other chemicals to which

a person may be exposed to either intentionally, accidentally or unknowingly through daily life (Ritter *et al.*, 1995). Induction will increase elimination rates, whilst conversely inhibition will reduce the rate of elimination and promote retention of the parent compound within the body. Consequently, prediction of the most prominent form likely to be encountered in the environment is made increasingly difficult.

While the primary function of metabolism is to remove pharmaceutical compounds from the body, conversion by Phase I & II reactions may yield two ultimate outcomes. The first and more favourable of these is that the drug in question is rendered pharmacologically inactive and therefore, should it enter the environment it should be no major cause of concern. The second more worrying scenario is that metabolism converts the pharmaceutical compound into a more potent or toxic form, (through either pro-drug activation or parent compound conversion). Examples of more potent metabolites include the conversion of codeine and heroin into morphine whilst common drugs used in high quantities such as paracetamol are known to have highly toxic metabolites such as N-acetyl-p-benzo quinone imine (Rang *et al.*, 1995).

The treatment of wastewater

The discharge from households and industry into drains and sewers is referred to as wastewater. Wastewater is on average >99.9% spent water with the other 0.1% comprising of dissolved and suspended solids (Wastewater Treatment Principles and Regulations, 2006). The actual composition of wastewater is highly variable. Likely components include microorganisms including pathogens, organic material, inorganic nutrients such as nitrogen and phosphorous compounds and metals (Henze, 2002). Each of these components may exhibit their own unwanted effects, if wastewater were discharged directly into ground and surface waters. ln an attempt to reduce the threat of pollution, wastewater usually receives some form of treatment before final discharge into the environment. In South Africa, the treatment of wastewater is governed by the Environmental Protection Agency Act of 1992 and more so by the 'Urban Wastewater Treatment Regulations 1994' passed to enact into South African law EU directive 911271EEC (Environmental Protection Agency, 1997). Under Section 85 of the 1992 Act, discharges to sewers must be monitored and are licensed under the Integrated Pollution Control system to protect the receiving treatment plant and the general aquatic environment in the long run (Environmental Protection Agency, 1997). A schematic of wastewater treatment processes is depicted in Figure 1-6.

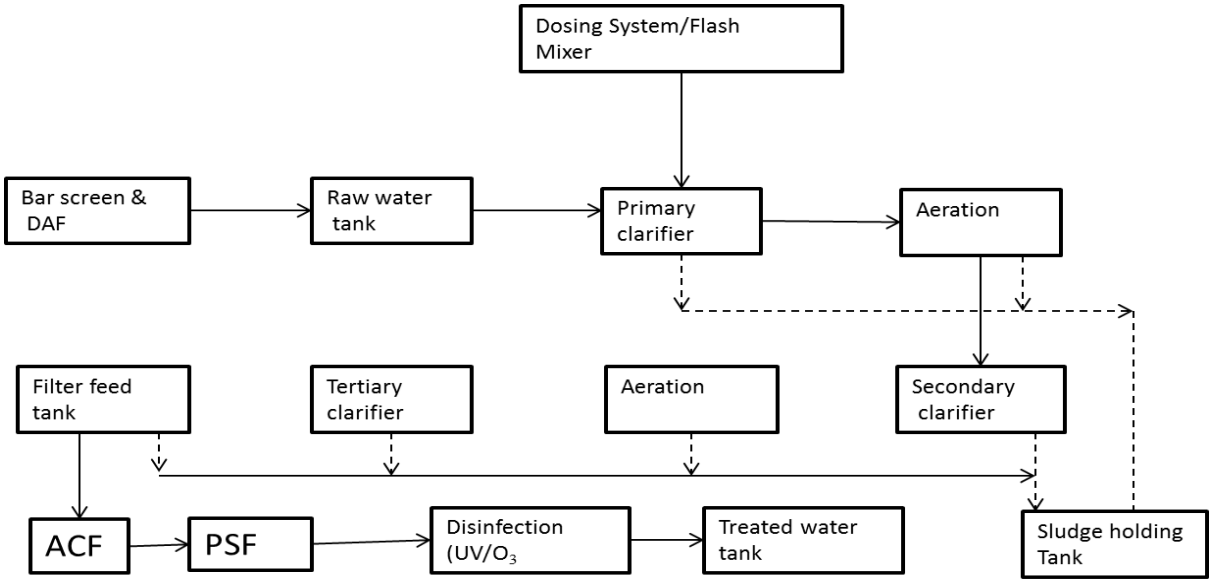


Figure 1-6: An overview of wastewater treatment (Adapted from (http://www.saitreat.com/images/effluent.png))

Wastewater treatment is a stepwise process of physical, biological and chemical means, designed to remove the aforementioned wastewater components and protect the effluent receiving water body. Physical processes are usually involved in the preliminary and primary treatment stages.

Preliminary treatment involves the filtration of influents to remove debris and large particles. The screened influent then passes into holding basins where the wastewater is held for sufficient periods of time to allow solids to settle to the bottom of the basin, while organic matter such as oils, fats and greases float to the top. Solids may settle out in a variety of ways depending on their physical properties, i.e. size and density, formation of associated masses of particles or compression; whereby settling particles drag other dissolved solids downwards (Environmental Protection Agency, 1997). Both layers can then be physically removed before the next process occurs. Biological treatment of wastewater occurs during the secondary stage, a process that is generally referred to as activated sludge treatment. Activated sludge consists of a complicated ecosystem of microorganisms ranging from heterotrophic and facultative bacterial species of Achromobacter, Arthrobacter, Citromonas, Flavobacterium, Nitrobacter, Nitrosomonas and Pseudomonas to higher protozoa such as Amoeba, Opercularia and Trachelophyllum and also rotifers and nematodes (Gray, 1989).

Activated sludge treatment involves the mixing of a concentrated microbial population with wastewater under aerobic conditions, in order to provide both oxygen and a carbon source

necessary for microbial respiration. Such conditions encourage high rates of microbial growth and consequently increased rates of microbial respiration, leading to a reduction in the quantity of organic matter present within the wastewater (Gray, 1989). Bacteria account for the highest proportion of microbes within the sludge and as bacterial cells grow, they may produce a slime layer surrounding the cell wall. The slime layer imparts an absorptive surface onto the bacterial cell, allowing for the formation of flocculated agglomerations of microbes, commonly referred to as 'flocs' (Prescott *et al.*, 2006). The forming floc surface also absorbs colloidal and suspended matter; ionic substances may also be absorbed due to interaction with oppositely charged biomolecules within the bacterial cell wall (Gray, 1989). Activated sludge treatment is a dynamic process as bacteria within a floc assimilate and utilise the adsorbed material. This will result in free sites on the surface of the floc capable of adsorbing more and more of the wastewater matrix.

A crucial factor in this process is the treatment time, also known as the hydraulic retention time, spent within the aeration tank in order to allow sufficient microbial activity. If the treatment time is not long enough, little organic material will be removed. The ecological make up of activated sludge is another important factor in maintaining a viable process. Protozoa species, aid the treatment process by feeding off the bacterial populations thereby preventing the bacteria from reaching excessive lag phase numbers. Protozoal feeding also helps with the removal of suspended matter (Gray, 1989). The final process in the sludge treatment is clarification, which basically is liquid-solid separation whereby the flocculated biomass is allowed to settle out of solution yielding a clarified effluent (Environmental Protection Agency, 1997). The effluent may then be subjected to tertiary treatment or be discharged into a receiving water body. The remaining sludge is either reintroduced, into the aeration tank as bacterial inoculum or is itself inactivated and disposed (Environmental Protection Agency, 1997).

Tertiary treatment of wastewater may be physical or chemical in nature. The objective of tertiary treatment processes is to remove non-biodegradable organic materials, metals and nutrients present even after the primary and secondary processes. Chemical methods are used for the removal of eutrophication nutrients, i.e. nitrates and phosphates. Phosphates can be precipitated out of solution by the addition of calcium or iron (Prescott *et al.*, 2006), while nitrates can be converted to volatile ammonia at high pH which is easily purged from solution by aeration. Nitrates may also be reduced at low pH to nitrogen gas or nitrous oxides (Prescott et al., 2006). Disinfection to inactivate any residual microbes, particularly

pathogens may also be required and practices such as chlorination may also aid with the removal of some pharmaceuticals (Kumar et al., 2006; Glassmeyer & Shoemaker., 2005; Bedner & MacCrehan., 2006; and Doll & Frimmel., 2005). Non-biodegradable organics may be subjected to advanced oxidation using ozone, whilst metallic elements maybe precipitated out of solution by reaction with an appropriate chelating agent (Eilbeck & Mattock., 1987).

An important factor in determining the performance of the treatment process is the measurement of dissolved oxygen concentration of wastewater influents and effluents. The most common measurement performed is that of biochemical oxygen demand (BOD) which measures the quantity of oxygen required by microbes for organic matter consumption (Environmental Protection Agency, 1997). Each treatment stage is designed to reduce the BOD of the wastewater, with the highest proportion as expected being removed by the activated sludge process. The more efficient the treatment process, the lower the expected BOD of the effluent.

The behaviour of pharmaceuticals in waste water treatment plants (WWTPs).

Richardson and Bowron (1999) proposed three possible outcomes for pharmaceutical compounds during the treatment of wastewater; mainly full degradation or partial degradation or persistence and discharge into the receiving environment with the treated effluents. The two most probable means for the removal of pharmaceuticals in wastewater treatment plants include microbial degradation either to produce compounds of lower molecular weight or ideally complete metabolism into CO_2 and H_2O, or the sorption of pharmaceuticals to particles and solid matter that can be removed by filtration or settling (Daughton & Ternes, 1999). In 1996, Rogers reviewed the behaviour of many classes of organic contaminants including some pharmaceuticals in sewage sludge (Rogers, 1996). At the time, information concerning the behaviour of pharmaceuticals was mostly speculative due to lack of suitably sensitive analytical methods, however, it was suggested that the presence of pharmaceutical compounds need not be a cause of concern and it was acknowledged that many compounds, mostly antibiotics, were readily biodegradable (Rogers, 1996).

Following his investigation on the occurrence and behaviour of pharmaceuticals in wastewater treatment plants, Ternes (1998) reported the presence of a multitude of drug residues from many pharmacological classes in the influent, effluent and receiving water of a municipal treatment plant near Frankfurt in Germany. By determining the difference between

the concentrations detected in the plant effluent and influent, the overall removal efficiency of the treatment process was estimated. It was reported that on average, less than 60% of the detected drug residues were removed, however, some compounds showed particularly low removal, e.g. the antiepileptic drug carbamazepine and clofibric acid, a metabolite of many lipid lowering agents and these compounds were ubiquitously present in the aquatic environment as a result (Ternes, 1998). Ternes also examined the presence and behaviour of highly polar and ionic compounds used as x-ray contrast media in the treatment plant, and discovered that such chemicals were not removed during treatment and passed freely through the plant contaminating the receiving waters (Ternes & Hirsch, 2000). Other studies conducted have reported similar findings both in the United Kingdom (Kanda *et al., 2003*) and Spain (Carballa *et al.,* 2004).

Carballa *et al.* focused on sampling at each stage of the treatment process in an attempt to ascertain which treatment step provided the highest rate of removal, (Carballa *et al.,* 2004). It was found that the degree of hydrophobicity of the analyte was important as more non-polar compounds were observed to adsorb onto the primary and secondary sludge with more polar analytes remaining in the water phase and therefore, passing unhindered through the treatment plant (Carballa *et al.,* 2004). Interestingly, levels of some compounds, e.g. 17P-estradiol, were observed to increase after secondary treatment suggesting the cleavage of Phase II metabolites during biological processes. Information regarding the behaviour and removal of pharmaceutical compounds in wastewater treatment plants is of great importance when attempting to estimate the loading of such compounds into the environment. For example, Fischer and Borland estimate that between 15 to 30 tonnes of active pharmaceutical ingredients are released into the environment surrounding Sydney, Australia on a yearly basis (Fisher & Borland, 2003) due to the insufficient treatment of wastes. With appropriate treatment, these quantities could be significantly reduced.

The microbial processes leading to the removal of pharmaceuticals during wastewater treatment have not been fully investigated. Studies conducted have shown that the microbiological usage of pharmaceutical compounds as carbon or nitrogen sources for metabolism occurs only in the absence of a primary substrate (Drillia *et al.,* 2005). However, an equal probability exists that microbes may show no preference and metabolise pharmaceuticals even in the presence of a primary substrate depending on the affinity and resistance of the microbes enzymes to such pharmaceuticals. The removal rate of some pharmaceutical compounds in WWTPs is observed to increase with increased residence time

((Kanda *et al., 2003*)); possible explanations for such an observation include the increased diversity of the microbial community with increasing sludge age or the ability of microbes to respond to limiting organic carbon availability (Ternes *et al.*, 2004).

Sorption to filterable solids during wastewater treatment has received more attention as it is generally a more understandable process than microbial degradation. Pharmaceuticals present in the aqueous phase of a WWTP may adsorb onto particulate and suspended material by hydrophobic interaction between nonpolar moieties of the molecule and lipid rich cell membranes of microbes or other agglomerations of fatty material on the sludge. Electrostatic interactions between oppositely charged groups on the pharmaceutical and the surface of microbes or particles may also be involved (Diaz-Cruz et al., 2003; Ternes et al., 2004). The distribution of pharmaceuticals between the aqueous phase and the solid phase is an equilibrium process, represented by solid water distribution coefficient; K_D, whereby;

$$K_D = \frac{C_{sorbed}}{C_{aqueous}}$$ **Equation 1-1(Adapted from (Diaz-Cruz et al., 2003)**

C_{sorbed} and $C_{aqueous}$ are the concentrations of pharmaceuticals in the solid and water phases respectively (Diaz-Cruz et al., 2003). K_D values allow for the prediction of whether a substance will show appreciable adsorption or will preferentially remain in the aqueous phase. K_D values for several pharmaceuticals have been experimentally investigated but found to be quite low thereby suggesting that the drugs chosen would be expected to exhibit negligible adsorption and therefore, microbial degradation plays a major part in the removal of pharmaceuticals in actual treatment plants (Ternes et al., 2004, Bouwer & MCoelhan, 2006).

Another study performed by Urase and Kikuta attempted to estimate both the sorption and degradation of pharmaceuticals during activated sludge treatment (Urase & Kikuta, 2005). They observed that the pH of the sludge played an important role in determining the adsorption onto the sludge. A theoretical model for the prediction of probable concentrations and subsequent removal rates was described by Khan and Ongerth (Khan & Ongerth, 2004) for the 'Top 50' prescribed pharmaceuticals in Australia. Parameters included within the model included data on pharmaceutical quantities used, metabolic and excretory data, chemical and physical properties for each compound and operating data for the types of

treatment plants involved. The model predicted removal rates ranging from 14% for the antibiotic roxithromycin to 99% for the antihypertensive irbesartan.

Two other items which were observed to affect the behaviour of pharmaceuticals during the treatment process were rainfall and the infrastructure of the plant (Ternes, 1998; Wolf *et al.,* 2004). Ternes reported that the removal of several pharmaceuticals, predominantly analgesics, was significantly reduced during periods of increased rainfall (Ternes, 1998). It was also observed that the rate of removal took several days to recover to its previous level. It was suggested that the reasons for decreased pharmaceutical removal may include a reduction in microbial activity or a change in the sorption and flocculation due to increased fluid flow though the plant. Wolf *et al.* investigated the effect of sewer infrastructure in the German city of Rastatt and its role in the release of pharmaceutical compounds to the environment (Wolf *et al.,* 2004). High concentrations of iodinated x-ray contrast media were detected in groundwater in the vicinity of sewer pipes, illustrating that significant leakage of untreated sewage was occurring underneath the city and also that measurable quantities of pharmaceutical compounds were being introduced to groundwater as a result. Iodinated x-ray contrast media were therefore suggested as an anthropogenic maker species for monitoring the presence of untreated wastewater in environmental waters.

Pharmaceutical disposal and landfill leachate

According to Slack et al., approximately 60-70% of all municipal waste produced in the developed world is disposed of in landfill sites (Slack *et al.,* 2005) and up to 5% of such wastes may contain hazardous materials originating solely from household use. However, as there is no legal definition of what constitutes hazardous household wastes and therefore, no enforced segregation procedures; it is difficult to accurately predict the quantities of hazardous substances being placed in landfill sites (Slack *et al.,* 2005). Slack *et al.* conducted a survey in the United Kingdom in order to gauge the amounts of hazardous materials held in households and the disposal routes for such products including expired or unused pharmaceuticals (Slack *et al.,* 2005). It was discovered in the case of pharmaceuticals, the public did not understand that pharmaceuticals constituted a hazard or how to safely dispose of such waste. Only 19% of expired or unused pharmaceuticals were returned to pharmacies for proper disposal with -50% being dumped in the bin and ultimately landfill and another -20% being flushed down the toilet (Slack *et al.,* 2005). Emissions from landfill sites are normally gases, airborne particles or more importantly leachate in the case of pharmaceutical

pollution. Leachate is expected to be complex and heterogeneous in composition depending on the types of wastes disposed in the landfill, contain both inorganic and organic constituents which pose a multitude of risks (Slack *et al.,* 2005). An important parameter in leachate analysis is the quantity of dissolved organic carbon (DOC), as it provides a surface for adsorption and also affects the mobility of metallic elements (Slack *et al.,* 2005).

Environmental processes and the fate of Pharmaceuticals and Personal Care Products (PPCPs).

Once pharmaceutical compounds enter the environment the question exists as to their fate, i.e. are they transported along the watercourse and diluted to such levels whereby their presence becomes negligible, or do they adsorb onto solids and accumulate over time leading to increased concentrations, or are they degraded or transformed into various other chemicals in the presence of sunlight? Few studies have been undertaken in an attempt to answer such questions, hence this study. Primary findings will be discussed in the following sections.

Environmental transport

Pharmaceuticals are predominantly introduced into the aquatic environment with treated wastewater at levels in the ngL^{-1} to the low pgL^{-1} range; the dilution of such residues, (and therefore, the dilution of the risk that they pose) depends upon the volume of the receiving water body and its ability to adequately disperse such chemicals. Ashton and colleagues (2004) investigated the introduction of drug residues into surface water from treatment plants in the United Kingdom. Samples of surface water were collected upstream and downstream of the plant along with the discharged effluent. In four instances, pharmaceuticals were detected prior to the treatment plant; the highest detection was the analgesic ibuprofen at a level of 181 ngL^{-1}. It was suggested that the detection of drug residues before the plant indicates that these chemicals were transported over a long range and therefore, have adequate stability to survive in the aquatic environment (Ashton *et al.,* 2004). A statistical analysis of the concentrations of pharmaceuticals detected in the discharged effluent and receiving surface water was also performed and it was found that a positive correlation existed between the two, i.e. the levels of pharmaceuticals detected in surface water is a 'diluted' reflection of the quantities present in effluent which in turn is a reflection of overall usage of those particular pharmaceuticals (Ashton *et al.,* 2004).

Other studies conducted have also illustrated that pharmaceuticals can undergo long range transport in the aquatic environment. Thomas and Hilton (2004) detected fourteen

pharmaceuticals in British estuaries of the Thames, the Tyne, the Mersey, the Tees and Belfast Lough and suggested that the detection of pharmaceutical analytes in estuaries is a result of contaminated surface water infiltration. Two separate studies were conducted to determine pharmaceutical residues in the North Sea (Wiegel *et al.*, 2002, Buser *et al.*, 1998). The North Sea is a particularly sensitive water mass which accepts rivers from the United Kingdom, Norway, Sweden, Denmark, Germany, Holland, Belgium and France. Clofibric acid was detected in both studies; it was observed that a concentration gradient existed from the mouth of certain rivers, in particular the Elbe, to the open sea and that levels detected were relatively stable over a considerable period of time and comparable with other "classic" pollutants such as Lindane (Wiegel *et al.*, 2002, Buser *et al.*, 1998).

A detailed study of the river Elbe in Germany was conducted by Wiegel *et al* (2004). It was found that the river was heavily polluted with pharmaceutical residues due to the large number of WWTPs discharging into the river. Transport of pharmaceutical residues can be observed with increasing concentrations being detected with distance from the rivers source to its exit; approximately 700 km (Wiegel *et al.*, 2004). Levels of clofibric acid were detected in the rivers plume into its North Sea estuary and the authors conceded that the river is a significant source of clofibric acid in to the monitored marine environment. The stability of pharmaceuticals in the environment is significant, however, it is also acknowledged that due to a 'steady state' of introduction, levels entering the environment are sufficient to replace those being removed (Bendz *et al.*, 2005). Pharmaceutical residues therefore lend themselves as appropriate marker species for tracking the transport and dilution of wastewater in the environment (Clara *et al.*, 2005).

Photochemical fate of pharmaceuticals

Knowledge concerning the fate of pharmaceutical compounds in the natural environment is essential when attempting to quantify the risk that they pose. Pharmaceuticals may be subject to both biotic processes, e.g. biological transformation and abiotic processes e.g. hydrolysis, photolysis or sorption in aquatic systems. Of the above processes, studies have shown that photolysis of pharmaceutical compounds in aquatic systems, is significantly more important than other biotic and abiotic processes (Buser *et al.*, 1998). Two distinct pathways exist by which photolytic reactions may occur; direct photolysis, wherein a molecule upon the absorption of light becomes unstable and decomposes or indirect photolysis, wherein molecules interact with the reactive intermediate of another species produced by its

absorption of light (Andreozzi *et al.*, 2003; Lam *et al.*, 2005). Research on the photochemical fate of pharmaceutical chemicals is limited, with only a handful of papers on the subject published to date.

The presence of antibiotic compounds in the environment has caused concern due to the possible development of microbial antibiotic resistance. Turiel and collaborators (2005) investigated the photochemical fate of two commonly used and potent quinolone and fluoroquinolone antibiotics; oxolinic acid and ciprofloxacin respectively. It was noted that ciprofloxacin degraded much faster than oxolinic acid upon irradiation. The matrix in which the experiment was performed also played an import role as humic material was observed to decrease the rate of photo degradation (Turiel *et al.*, 2005). Results suggested that quinolone antibiotics, due to their much slower rate of degradation may pose more of a risk to the development of microbial resistance than fluoroquinolones. However, attempts to identify the photoproducts of ciprofloxacin revealed that the compounds still contained the active centre of the molecule. lt was then suggested that in attempting to perform environmental risk assessments, both the parent molecule and degradation products should be considered. lsidori and colleagues (2005; 2006) recommended the same approach as they investigated the ecotoxicity of both the anti-inflammatory naproxen and its photoproducts and the diuretic furosemide and its photoproduct and observed that the photoproducts were more toxic than the parent pharmaceuticals.

Sorption and Mobility in Solid Matrices

Pharmaceuticals may adsorb onto solids during the treatment of wastewater and as such, be removed with the sludge. While this process attenuates the levels of pharmaceuticals being discharged along with the treated effluent, a problem exists with the disposal of sludge, which as a re is likely to contain quite significant quantities of pharmaceuticals, e.g. a German study found levels of Triclosan at approximately 50 ngL^{-1} in WWTP effluent and approximately 1200 ngg^{-1} in the corresponding sludge (Bester, 2003). Probability exists that pharmaceuticals may leach out under suitable conditions. A similar problem exists with veterinary medicines that may be introduced into the environment through the spreading of treated animal wastes on lands as fertiliser, thereby contaminating soils, groundwater and surface water through overland flow (Boxall *et al.*, 2002; Kay *et al.*, 2005).

The sorption of drug residues in the environment leads to increased localised concentrations of those particular analytes. One particular analyte known to accumulate is the antibacterial

agent Triclosan. It has been demonstrated that at high pH Triclosan is readily photo degraded, but the associated form of the molecule is relatively stable, so much so that it has been shown to bioaccumulate in fish exposed to treated wastewater effluent and even in human milk (Adolfsson-Erici *et al.,* 2002). Nevertheless, conflicting reports were published by the manufacturers of Triclosan who claim that the molecule is not persistent (Sabaliunas *et al.,* 2003). The sorption of veterinary pharmaceuticals to soils was reviewed by Tolls (2001) who reported that traditional approaches to describe sorption such as K_D, K_{OW} and K_{OC} that cater solely for sorption through hydrophobic interaction do not properly portray the sorption behaviour of drugs in soils. These parameters fail to account for hydrogen bonding, ion exchange and chelation, which are more important sorption and retention mechanisms for pharmaceuticals likely to be charged at soil pH, (Tolls, 2001). Christian *et al.* determined antibiotics in German soil, (Christian *et al.,* 2003) and observed that one particular sulphonamide; sulfadimidine, was stable for long periods of time after application. The potential for pharmaceuticals bound to soils to leach and contaminate groundwater has become the focus for many studies. Oppel *et al.* investigated the leaching behaviour of six drugs from two different soil columns of different pH and organic content (Opell *et al.,* 2004). The study reported that carbamazepine, diazepam, ibuprofen and ivermectin were retained on both soil columns, while clofibric acid and iopromide were determined solely in the leachate. Using radio-labelled standards, it was possible to determine the depth penetration of the pharmaceuticals in the column.

Kay and collaborators (2005, 2005) published two studies concerning the mobility of three veterinary antibiotics in soil treated with slurry. Both studies detailed that oxytetracycline and the macrolide tylosin do not leach from soil with the application of slurry, but the sulphonamide; sulfachloropyridazine was highly mobile, with quantitative recovery of the applied quantity used during the study. The high mobility of the sulfonamide was attributed to macropores in the soil structure that allowed for unhindered transport to drainage systems and ultimately surface water (Kay *et al.,* 2005). The application of slurry was observed to cause an increase in soil pH but tillage of the soil prior to application was found to remove the risk for all the studied compounds Kay *et al.,* 2004). Similar findings to the above studies were reported by Drillia *et al.* (2005). Additionally it was observed that the soil type and channels within the soil were important. , Simulated rainfall events were performed and it was observed that the flow and volume of rain affected the adsorption and the mobility of the drug through the soil. The higher the volume and flow, the less drug adsorbed, suggesting

that high concentrations of pharmaceuticals may be released to surface and ground water during intense rainfall, although the increased volume will aid with dilution (Drillia *et al.*, 2005)

The processes affecting the concentrations and fate of pharmaceuticals in the environment are highly complex. Pharmaceuticals have adequate stability and can be transported over considerable distance through the water course. Soils may adsorb some pharmaceutical residues, however, such an effect was observed to concentrate levels in the uppermost topsoil layer (Golet *et al.,* 2003). Rainfall can affect sorption and lead to increased introduction of drug residues into groundwater. Photolysis is an important process in the removal of pharmaceuticals from the natural environment, but is itself a complicated process as many transformation products may still contain active groups and express pharmacodynamic effects.

Assessing the ecotoxicology and risk posed by Pharmaceuticals and Personal Care Products (PPCPs)

Perhaps the most worrying effect was reported by Schwartz *et al.* (2003) and Ohlsen *et al.* (2003) who detail the presence of antibiotic resistant bacteria in biofilms on the distribution systems of wastewater, surface water and drinking water. Bacteria carrying resistant genes were found in drinking water. This is unusual as the resistance genes detected are normally associated with enterobacteria detected in wastewater and WWTPs. Both studies suggested that the transfer of drug resistant genes and plasmids via bacterial conjugation is extremely likely and therefore, the threat of further emergence of antibiotic and drug resistant microbes is increased due to the exposure to pharmaceuticals in the environment (Schwartz *et al.,* 2003; Ohlsen *et al.,* 2003).

Pharmaceuticals and Personal Care Products (PPCPs) and drinking Water

The presence of pharmaceutically active compounds in surface and groundwater poses a troubling concern; as such systems are often used as supply for the production of potable water. Although the previous section focused on the eco-toxicological risk caused by pharmaceutical residues to exposed marine organisms, the presence of drugs in drinking water can provide direct entry into the human body. To-date very few reports have been published confirming the presence of pharmaceuticals in drinking water. The reason for such a small number may be twofold: firstly the concentrations present may be too low and therefore, beyond the detection limits of most current analytical methods or secondly,

pharmaceutical residues may be efficiently removed during drinking water treatment (Jones *et al.*, 2005)

Although the levels of drugs present in drinking water are extremely low, the risk posed to humans through continual exposure needs to be assessed. Webb *et al.* (2003) estimated the lifetime exposure of the average person who drinks two litres of drinking water per day every day over the course of a seventy year lifespan for sixty pharmaceutical compounds. Assuming the worst case scenario, the lifetime ingestion of pharmaceutically contaminated drinking water was less than the daily recommended dose for the vast majority of compounds investigated (Webb *et al.*, 2003). Similar findings were recently reported by Schwab et al. who employed an analogous model (Schwab *et al.*, 2005). In the case where the lifetime exposure was greater than a recommended daily dose, (e.g. for ethinylestradiol and the x-ray contrast medium iopromide) the calculation could be refined and corrected to include metabolic and WWTP removal data. The question of the presence of antineoplastic and cytotoxic pharmaceuticals was also raised, because although these chemicals are used in the treatment of cancer, many are inherent carcinogens themselves and therefore, a significant risk is posed by any level, even the minutest exposure (Webb *et al.*, 2003). The general conclusion from both studies was that the risk is indeed low. However, further investigations need to be undertaken to assess the risk posed to more vulnerable groups of society such as infants, the elderly, dialysis patients etc. (Jones *et al.*, 2005).

Prevention is better than Cure

As pharmaceuticals are continually being introduced into the environment along with treated wastewater effluents, the problem appears to be persistent. Finding a solution will not be an easy task as the benefits of medicine outweighs environmental presence and risk. That is, the advent of environmentally friendly pharmaceuticals is not going to happen in the short term, considering the amount of time and money required for drug development. A concept introduced in Sweden however may provide some promise. The concept ranks pharmaceuticals based on the threat they pose to the environment, allowing doctors to prescribe, or patients to choose, more environmentally friendly treatments from existing medicines (Larsen *et al.*, 2004). The use of the 'precautionary principle', which can be interpreted as the lack of scientific facts concerning a potential hazard not justifying inaction to prevent such a hazard (Larsen *et al.*, 2004), is inappropriate of pharmaceuticals for the above reasons. It appears that finding an acceptable solution for the prevention of

pharmaceutical compounds contaminating the aqueous environment through non-point source pollution is by no means an easy task and therefore, an area that requires significant research.

1.3 Problem statement

Pesticide and PPCPs residues may reach the aquatic environment through nonpoint and point pollution sources of natural waters. Although significant advances have been made in controlling point-source pollution, little progress has been made regarding the nonpoint-source pollution on river banks due to increased pollution threats from industrialisation and urbanisation on the river banks, inherent variability and multiplicity of origins of nonpoint-source pollution. Identifying possible pollution effects on the Kat River is not only urgent but necessary as no study has been conducted yet in Kat River.

1.4 Hypothesis

We therefore hypothesise that urbanisation and industrialisation on the Kat River banks have a major impact on downstream farming activities and/or downstream water health and security.

1.5 Aim

This current study is aimed at identifying the level of pesticides and other water contaminants (pharmaceuticals and personal care products) polluting the Kat River and the effects these may have on farms/communities occurring on its banks.

1.6 Objectives

- To identify the possible pesticide contaminants and their relative concentration at various sampling points on the Kat River water.
- To identify the possible pharmaceuticals and personal care products (PPCPs) contaminants and their relative concentration at various sampling points on the Kat River water.
- To identify possible sources of the contaminants identified in objective one and two above.
- To identify possible pesticide contamination in fruits and vegetables and their relative concentration from farms on the Kat River.

2 CHAPTER TWO

ANALYSIS OF PESTICIDES

2.0 Introduction

2.1 Pesticide water contamination

There has been substantial increase in the use of pesticide to increase crop yield due to a rapid increase in population growth, which in turn called for an increased food production to cater for the growing population. Pesticide residues in food products became a huge concern which required better and transparent agricultural practices. Many consumers were exposed to pesticide residues even at low concentration levels through uptake of fruits and vegetables. Therefore there was need to monitor these concentration levels of pesticide residues and the potential risk they can pose to human health. Paya and colleagues (2007) reported that there were increased violation rates in the maximum residue limits (MRLs) and increased incidences where pesticides were misused and hence many consumer organisations have taken their stand to fight governments and food industries to take into consideration pesticide use and the potential health effects pesticide residues can pose even when consumed in minute quantities (Paya et al., 2007).

Many nutritionists encourage people to increase their uptake of fruits and vegetables in order to help prevent chronic diseases but it has been seen that this may come at huge risk as increased uptake of fruits and vegetables may also mean increased exposure of individuals to pesticide residues (Drouillet-Pinard et al., 2010). Exposure to organo-pesticides (OP) occurs in many various ways and this varies with individuals. However, the major route through which many people are exposed is through dietary intake especially with children (CFSAN FDA, 2003).

Between 2002 and 2003, the Centers for Disease Control conducted epidemiologic studies including markers of exposure in biological samples being measured as a way to estimate the absorbed dose in humans. Researchers have reported many incidences of pesticide exposure especially through intake of fruits and vegetables due to the increased use of pesticides (Urairat et al., 2010). Therefore it has taken awareness to most control authorities including

governments to put monitoring measures such as the MRLs and even tolerances as a way of protecting the general public's health and the environment (Barr & Needham 2002).

2.2 Epidemiology and pesticides

Epidemiologist Roy Shore wrote "the single greatest weakness of epidemiologic risk assessment is that individual quantitative exposure information is very often limited or missing in occupational and environmental studies" (Shore, 1995). Through many epidemiological studies, it has been shown that there is a direct relationship between parental exposures to pesticides and children who are born with leukemia (Leiss & Savitz, 1995).Also studies have shown that neurological diseases are associated with chronic exposures to pesticide application (Denise *et al.,* 2003). Parkinson's disease (PD) is a disease of the nervous system that is characterized by progressive tremor, rigidity, and postural instability. It has been proposed that many exogenous toxicants, including pesticides, might be involved in the etiology of PD (Terry *et al.*, 2006). Chronic exposure to pesticides may also contribute in delayed neurophysiological processes that are involved in early stages of selective attention and late stages of sensory information processing such as stimulus evaluation and updating of working memory (Dassanayake et al., 2009).

2.3 Targeted research area

There is a growing need to increase the global food supply, putting pressure on farmers to maximise yields from their crops which has increased the use of pesticides, fungicides, and herbicides on crops in recent decades to aid in preventing crop spoilage from insects, weeds, microbes, and fungi and improve the post-harvest storage of the crop. Unfortunately, these pesticide compounds, and their metabolites and by-products, enter the food supply and food chain through non-point source pollution and are consumed by the general public. With over 1000 pesticides currently available for use and with increased regulation by many governments, the detection and identification of compounds across multiple classes has grown in demand.

2.4 Materials and methods

Chemicals

Methanol, water and acetonitrile of LC–MS quality was purchased from Sigma Aldrich (USA), while the formic acid (analytical grade) was purchased from Agilent, (USA). The QuEChERS kit (a mixture of MgSO4 and sodium acetate and primary secondary amine, PSA, tubes) was purchased from Waters (USA). The LC–MS column was purchased from Agilent (USA). All certified standards, APCI Negative calibration solution and APCI Positive calibration solutions were purchased from Separations (SA). All reagents were freshly prepared in LC/MS grade water or solvent before use.

Targeted pesticides

The most commonly used organophosphates and non-organophosphates pesticides used in the spray program of farmers in the Kat River were considered. The following list include the targeted pesticides studied: Spinosad ; Avermectin; Benzimidazole ,Dichlorprop-P; Buprofezin; 8-Dodecen-1-yl acetate; Chlorpyrifos; Granulovirus; Spinetoram; Gibberellic Acid; Azoxystrobin; Helicoverpa armigera nucleopolyhedrovirus; Imadicloprid; Fenpyroximate; Spirodiclofen; Mancozeb; Cypermethrin ; Pyriproxyfen; Glyophosate and Methidathion.

2.5 Sampling and sample preparation

Water sampling for pesticide analysis

Water samples (500ml) were collected using amber glass bottles. 15 sample points along the Kat River, ranging from a point as close to the source as possible to a point just before it joins the Great Fish River were used. The samples collected from the source were used as the control in each of the experiments, the assumption being the closer you get to the source, the less contaminated the water would be. Points were selected where the Kat River crosses the R67 or on farms where the river was accessible using farm roads. Samples were collected from October 2013 to November 2014.See Table 2-1 for pesticide sample collection points and coordinates and Figure 2-16 to Figure 2-18 for Kat River coverage.

The direct injection method was used for introducing the samples for analysis with the liquid chromatograph coupled to tandem mass spectrometry. The water samples were first

centrifuged at 13400Xg before being filtered through filtration cellulose acetate membrane filters (0.45um) and loaded directly into 1.5ml sample vials. All water samples were brought to the laboratory on ice and analysed immediately.

Table 2-1: Pesticide sample collection points and coordinates

Sampling point and description	Coordinates
Point A (Source)	(-32.538729;26.765033)
Point B (Upstream Seymour WWTP)	(-32.5486623;26.764190)
Point C (Downstream Seymour WWTP)	(-32.561004;26.771847)
Point D (Kat River Dam)	(-32.577643; 26.721951)
Point E (Before Balfour community)	(-32.555613;26.690156)
Point F (After Balfour community)	(-32.563859;26.77314)
Point G (Baflour farms)	(-32.578281; 26.679385)
Point H (Bridge)	(-32.585992; 26.685124)
Point I (Bridge)	(-32.605109; 26.668817)
Point J (Before Fort Beaufort town)	(-32.665139; 26.637741
Point K (Fort Beaufort town)	(-32.674099; 26.631819)
Point L (After Fort Beaufort town)	(-32.710738; 26.587230)
Point M ((Upstream Fort Beaufort WWTP)	(-32.772922;26.630673)
Point N (Upstream Fort Beaufort WWTP)	(-32.779291; 26.620856)
Point O (Further down the WWTP)	(-32.777643;26.645549)

Fruits and Vegetable sampling for pesticide analysis:

Different fruits and vegetable samples were collected from farmers on the river banks and taken to the laboratory for analysis. The vegetables were collected from small scale farmers using Kat river water for irrigation.

Pesticides were extracted from fruits and vegetables using QuEChERS (Quick, Easy, Cheap, Effective, Rugged and safe) method using the QuEChERS extraction kit (Waters) using the AOAC Method as per the supplier instruction manual. Briefly, 500g of sample was weighed and homogenized and transferred to 50ml tube where acetonitrile was added and shaken for a minute. Buffering salts were then added followed by centrifuging for 5 minutes at 13400Xg. The resulting supernatant was then transferred to the dispersive solid phase extraction clean

up tube, shaken and centrifuged. The resulting supernatant was then transferred to the 1.5ml sample vials, diluted with mobile phase and ran for analysis. The QuEChERS method allows for both extractions of pesticides from the samples and/or enrichment of the sample and sample clean up hence the product is ready for LC/MS analysis.

2.6 Instrumentation and chromatographic conditions

Liquid chromatography with mass spectrometric detection (LC/MS) was carried out using a 5600 AB SCIEX Triple TOF hybrid mass spectrometer (Applied Bio systems Sciex, USA) equipped with a high performance Agilent 1260 infinity liquid chromatography system and operated in both the positive and negative turbo ion spray (ESI) mode. The LC chromatography was fitted with a 4.6 x 50mm reverse phase column Proshell 120; EC-C18 with diameter 7µm or a 4.6 x 250mm ZORBAX SB-C18 with diameter 5 µm. Water with formic acid and acetonitrile were used as the mobile phases (Solvent A- Water with 0.1% formic acid; Solvent B- Acetonitrile with 0.1% formic acid).Bound compounds were eluted with a given gradient. The elution gradient was as follows: the mobile phase started with 5 % of Acetonitrile, which was increased linearly to 95 % in 10 minutes, and kept constant for 1 minute; finally, it was returned to the initial conditions in 0.5 min and kept constant for 5minutes giving a total run time of 17minutes. The column was equilibrated for 5 min, and flow rate was 0.5 mL min^{-1}.

TOF MS parameters were as follows: The Declustering Potential (DP) was 60V and Collision Energy (CE) was 35V with collision energy spread (CES) of ±35V. Product ion parameters were as follows: IonSpray Voltage Floating parameter (ISVF) 5500, Ion Source Gas 1 (GS1) parameter was 50 psi, Ion Source Gas 2(GS2) parameter was 50 psi and Temperature (TEM) was 600V.

An Agilent 1260 High Performance Auto sampler, with a 100µl syringe was used, the injection Volume was 200.µl, the samples were drawn and injected at a speed of 200 µl/min. Agilent column oven was set at 40°C for both right and left temperatures with a temperature tolerance of +/- 2°C.

2.7 Data analysis

Pesticide data analysis was done using three approaches which are the Target screen approach, Non-target screen approach and the retrospective (identification of pesticides not in the library) approach.

Target screen data Analysis approach

Target screening approach was used to identify and confirm the presence of other potential pesticide contaminants which were within the farmers spray regime for the season 2013-2104. The target screen approach is outlined below.

2.7.1.1 Step one

Full scan acquisition to get total ion chromatograms (TICs) of target analytes was done with the aid of the Peak View and Analyst Software (ABSciex) and the data obtained is shown in Figure 2-1

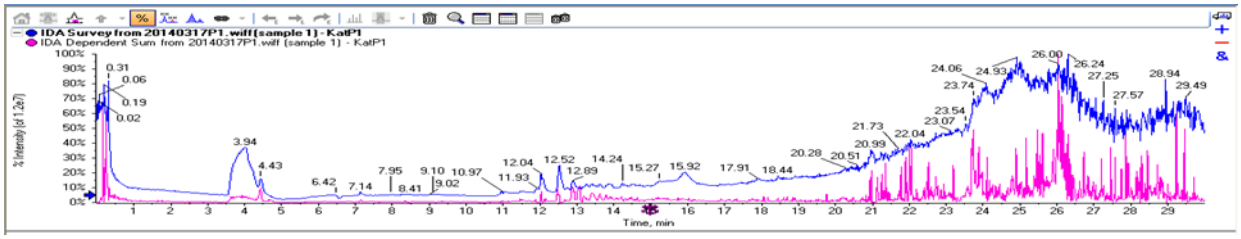


Figure 2-1: Total Ion Chromatograms obtained from one sample.

2.7.1.2 Step two

The elementary composition of the target compounds was obtained using the XIC manager. The XIC Manager allows you to manage large lists of compounds and perform automatic extracted ion chromatogram (XIC) calculations and review results operations. The results are displayed in the chromatogram pane as shown in Figure 2-2

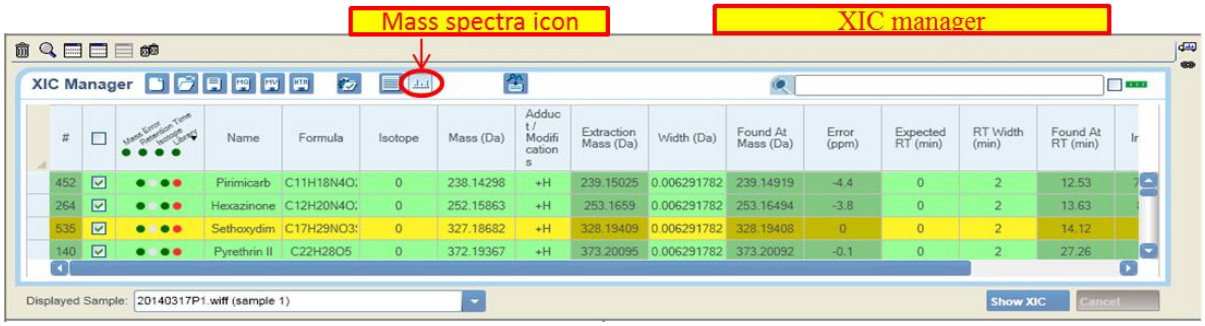


Figure 2-2: Chromatographic pane showing the retention time a particular peak was identified. This is extracted from the TIC.

2.7.1.3 Step three

The chromatogram showing the retention time is obtained by clicking on one of the identified compounds in the XIC manager and the results obtained are as shown Figure 2-3.

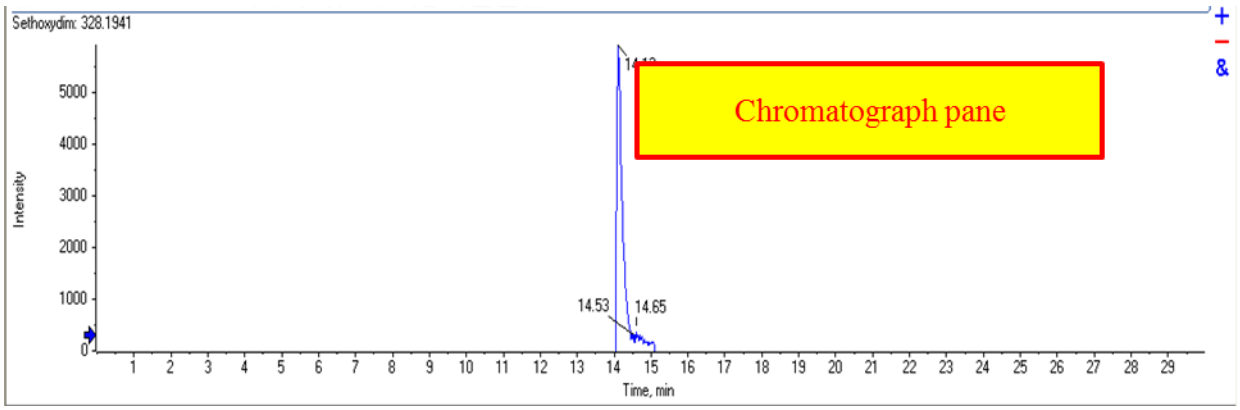


Figure 2-3: Chromatographic pane showing the retention time a particular peak was identified. This is extracted from the TIC.

2.7.1.4 Step four

The TOF-MS and Product ion of the identified pesticide compounds were obtained by simply selecting one identified compound in the XIC manager and clicking the MS spectra icon on the XIC manager menu bar (see Figure 2-2). The results will be displayed as shown in

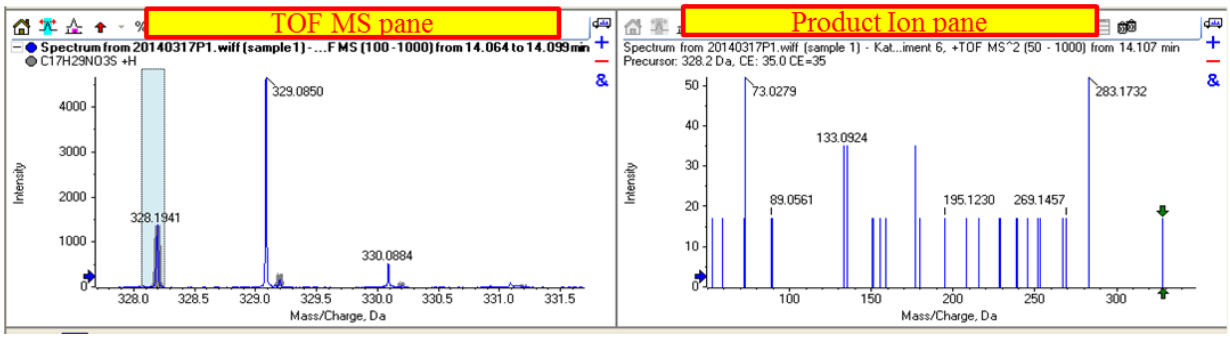


Figure 2-4: The TOF-MS pane (left) displays the mother peak and its mass found at specific retention time while the Production pane(right) displays the fragments of the breakdown of the mother Peak.

2.7.1.5 Step five

The ChemSpider website (www.ChemSpider.com) was used to obtain the structure of the identified compounds. This was done by simply loading the chemical formula obtained from the XIC Manager on the ChemSpider website; the structure of a respective compound can be obtained. A typical result displayed in Figure 2-5.

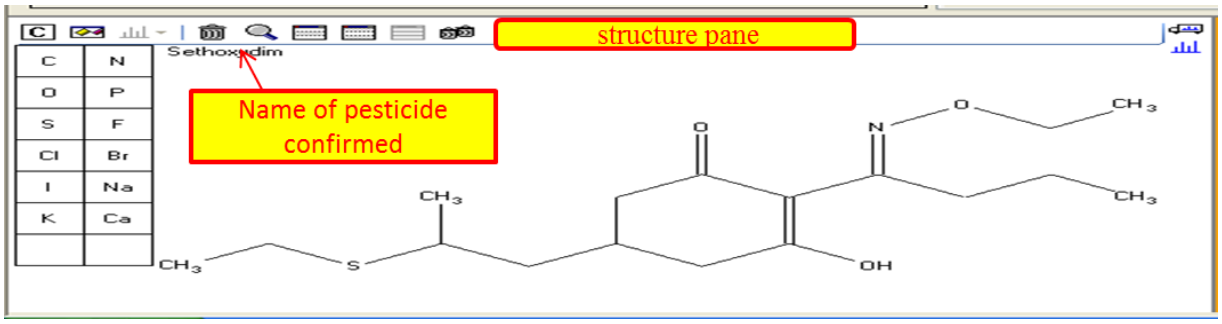


Figure 2-5: The structure of Sethoxydim obtained by submitting the chemical formula from the formula finder pane to ChemSpider website.

Non-target screen approach.

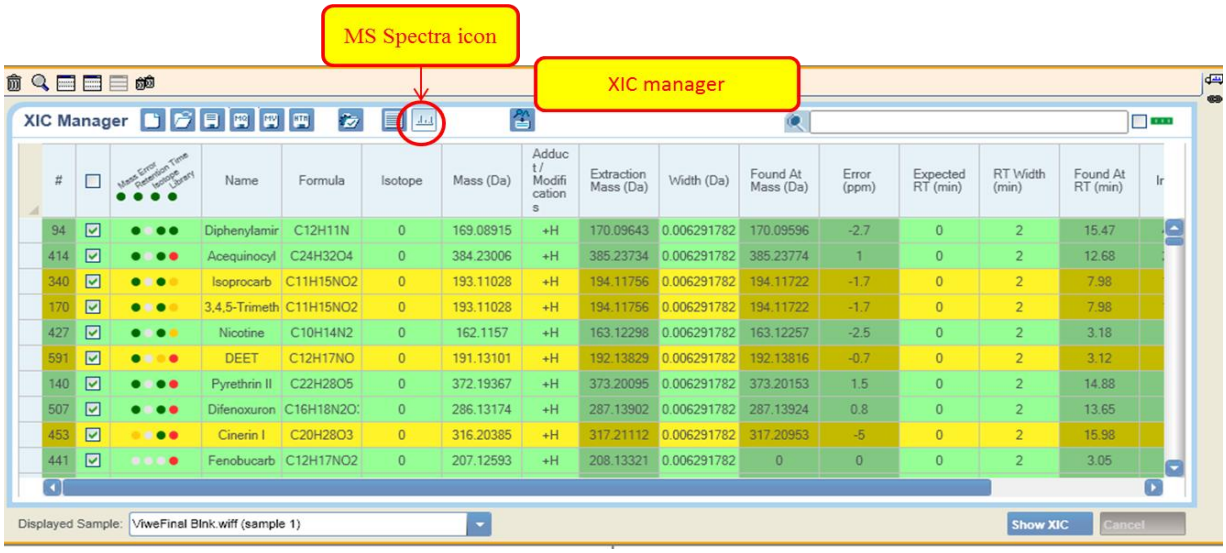
Non-target screening approach was used to identify and confirm the presence of other potential pesticide contaminants which outside the farmers spray regime for the season 2013-2014 but are found within the pesticide library. The non-target screen approach is outlined below.

2.7.1.6 Step one

The first step for non-target screen approach is a described in step 1 for target screen approach (See section 2.7.1.1).

2.7.1.7 Step two

The XIC Manager also performs automatic screening of samples, to identify non-targeted compounds. This is achieved by automatically searching the acquired TOF-MS/MS spectra in the pesticide library to assist compound identification as shown in Figure 2-6



#	Name	Formula	Isotope	Mass (Da)	Adduct / Modifications	Extraction Mass (Da)	Width (Da)	Found At Mass (Da)	Error (ppm)	Expected RT (min)	RT Width (min)	Found At RT (min)
94	Diphenylamir	C12H11N	0	169.08915	+H	170.09643	0.006291782	170.09596	-2.7	0	2	15.47
414	Acequinocyl	C24H32O4	0	384.23006	+H	385.23734	0.006291782	385.23774	1	0	2	12.68
340	Isoprocarb	C11H15NO2	0	193.11028	+H	194.11756	0.006291782	194.11722	-1.7	0	2	7.98
170	3,4,5-Trimeth	C11H15NO2	0	193.11028	+H	194.11756	0.006291782	194.11722	-1.7	0	2	7.98
427	Nicotine	C10H14N2	0	162.1157	+H	163.12298	0.006291782	163.12257	-2.5	0	2	3.18
591	DEET	C12H17NO	0	191.13101	+H	192.13829	0.006291782	192.13816	-0.7	0	2	3.12
140	Pyrethrin II	C22H28O5	0	372.19367	+H	373.20095	0.006291782	373.20153	1.5	0	2	14.88
507	Difenoxuron	C16H18N2O3	0	286.13174	+H	287.13902	0.006291782	287.13924	0.8	0	2	13.65
453	Cinerin I	C20H28O3	0	316.20385	+H	317.21112	0.006291782	317.20953	-5	0	2	15.98
441	Fenobucarb	C12H17NO2	0	207.12593	+H	208.13321	0.006291782	0	0	0	2	3.05

Figure 2-6: XIC manager pane displaying results of identified pesticides after running the TIC against a commercial pesticide library.

2.7.1.8 Step three

The TOF-MS and Product ion of the identified pesticide compounds were obtained by simply selecting one identified compound in the XIC manager and clicking the MS spectra icon on the XIC manager menu bar (see Figure 2-6). A typical result displayed in Figure 2-7.

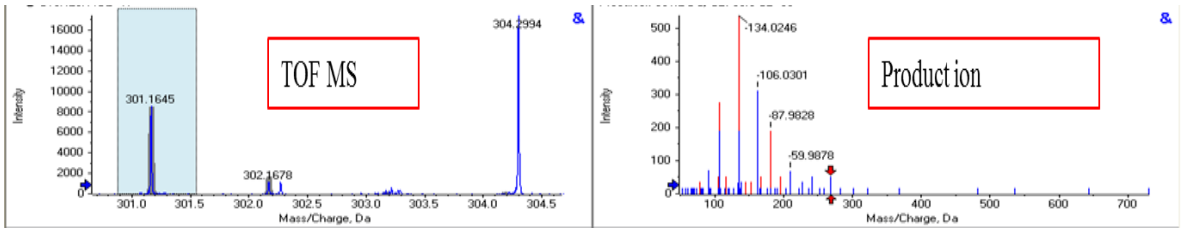


Figure 2-7: The TOF-MS pane (left) displays the mother peak and its mass found at specific retention time while the Production pane(right) displays the fragments of the breakdown of the mother Peak.

2.7.1.9 Step four

The Formula Finder software was used for the identification of the target analyte. The formula finder have two main functions which are: (1) To displays possible chemical formula that corresponds to the TOF-mass displayed in the TOF-MS pane depending on the elementary chemical composition one feeds into the software. The one with the lowest error displayed is ppm is usually the best match (2) to display the MS/MS details by basically displaying the number of fragments that corresponds to the Product ions in the Product ion

pane. Typical results displayed in Figure 2-8 and Figure 2-9 respectively. However further confirmation will still be required.

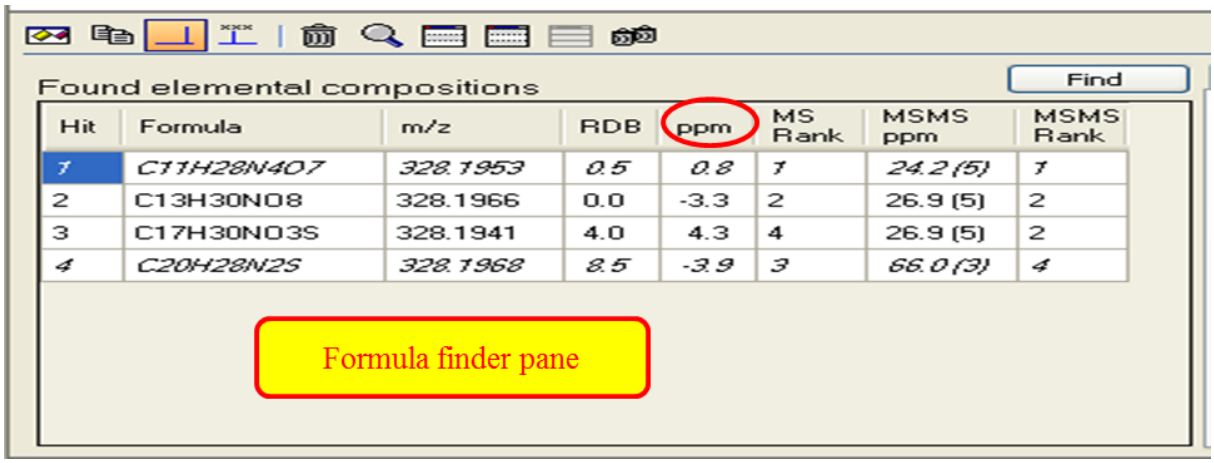


Figure 2-8: Formula finder showing possible chemical formula of the selected pesticide contaminant.

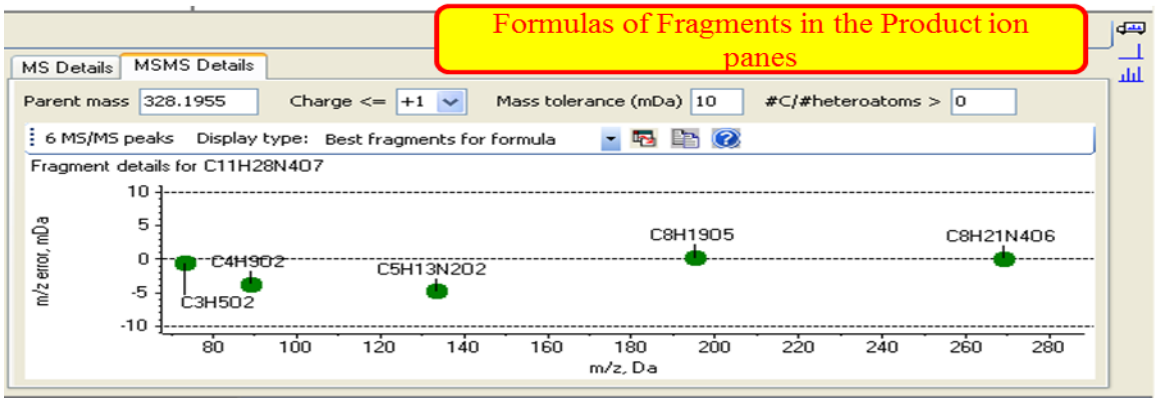


Figure 2-9: Formula finder pane showing formulas of fragments displayed in the Product ion pane.

2.7.1.10 Step five

The identification of the structure was done through the ChemSpider website. ChemSpider is a database that allows an easy access to over 28 million structures. By simply loading the chemical formula obtained from the formula finder pane on the ChemiSpider website, the structure of a respective compound can be obtained. A typical result displayed in Figure 2-10

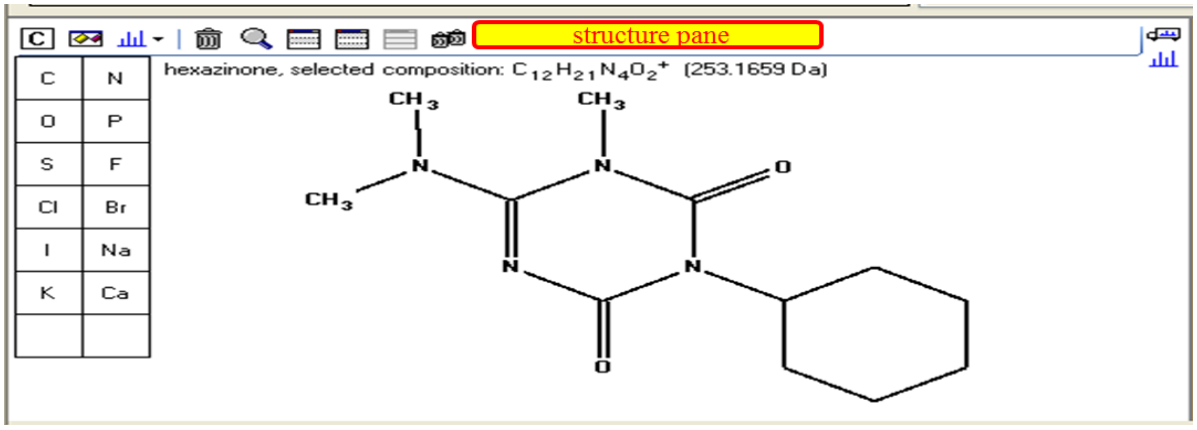


Figure 2-10: The structure of Sethoxydim obtained by submitting the chemical formula from the formula finder pane to ChemSpider website.

2.7.1.11 Step six

The confirmation of the compounds identified was achieved by the use of the Fragment pane Software by linking the structures to the MS/MS (product ion) of the selected m/z and to the fragment pane. The fragment pane software gives the confidence in the confirmation as it gives a percentage match of the structure and the product ions of that structure. The higher the percentage of match the higher the confidence matches. A typical result displayed in Figure 2-11.

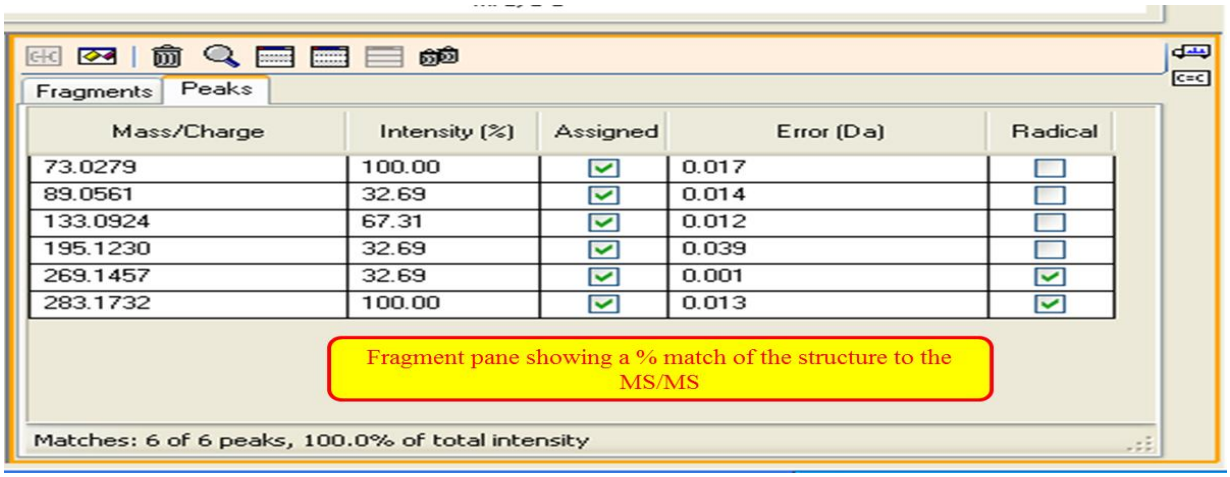


Figure 2-11: Fragment pane used for the confirmation of sethoxydim, with a 100% match of the structure to the MS/MS.

Retrospective screening (identification of pesticides not in the library) approach.

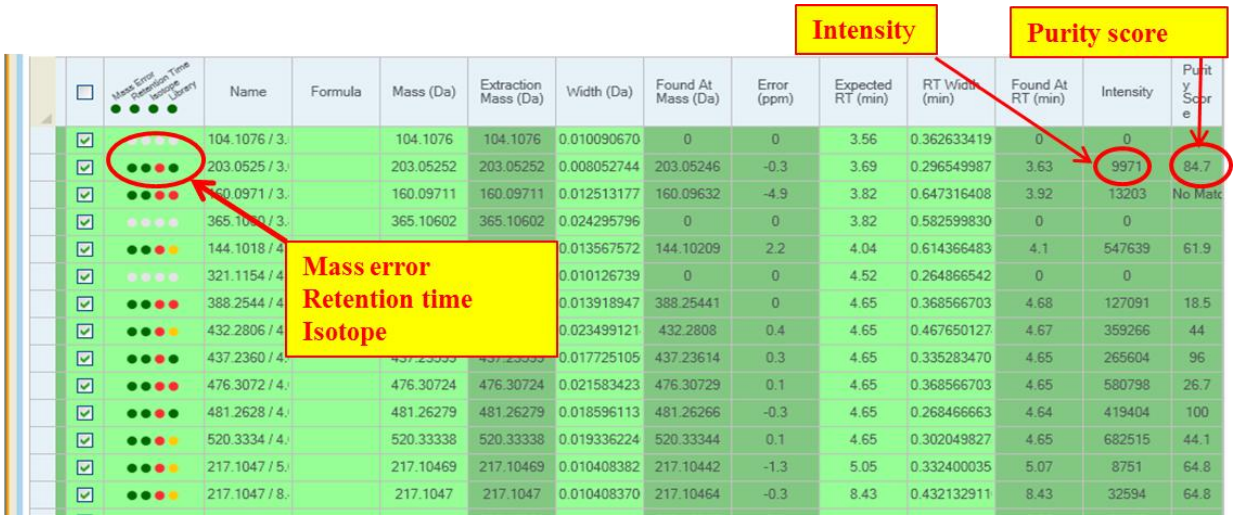
The presence of pesticide compounds which were not in the pesticide library was confirmed by using retrospective screening approach. The retrospective screen approach is outlined below.

2.7.1.12 Step one

The first step for non-target screen approach is a described in step 1 for target screen approach (See section 2.7.1.1).

2.7.1.13 Step two

The XIC Manager also performs retrospective screening of samples based on the presence of accurate mass molecular ion, characteristic fragments ions in MS/MS mode and isotopic mass. A typical example is displayed in



Name	Formula	Mass (Da)	Extraction Mass (Da)	Width (Da)	Found At Mass (Da)	Error (ppm)	Expected RT (min)	RT Width (min)	Found At RT (min)	Intensity	Purity Score
104.1076 / 3.		104.1076	104.1076	0.010090670	0	0	3.56	0.362633419	0	0	
203.0525 / 3.		203.05252	203.05252	0.008052744	203.05246	-0.3	3.69	0.296549987	3.63	9971	84.7
160.0971 / 3.		160.09711	160.09711	0.012513177	160.09632	-4.9	3.82	0.647316408	3.92	13203	No Matc
365.10 / 3.		365.10602	365.10602	0.024295796	0	0	3.82	0.582599830	0	0	
144.1018 / 4				0.013567572	144.10209	2.2	4.04	0.614366483	4.1	547639	61.9
321.1154 / 4				0.010126739	0	0	4.52	0.264866542	0	0	
388.2544 / 4				0.013918947	388.25441	0	4.65	0.368566703	4.68	127091	18.5
432.2806 / 4				0.023499121	432.2808	0.4	4.65	0.467650127	4.67	359266	44
437.2360 / 4.				0.017725105	437.23614	0.3	4.65	0.335283470	4.65	265604	96
476.3072 / 4.		476.30724	476.30724	0.021583423	476.30729	0.1	4.65	0.368566703	4.65	580798	26.7
481.2628 / 4.		481.26279	481.26279	0.018596113	481.26266	-0.3	4.65	0.268466663	4.64	419404	100
520.3334 / 4.		520.33338	520.33338	0.019336224	520.33344	0.1	4.65	0.302049827	4.65	682515	44.1
217.1047 / 5.		217.10469	217.10469	0.010408382	217.10442	-1.3	5.05	0.332400035	5.07	8751	64.8
217.1047 / 8.		217.1047	217.1047	0.010408370	217.10464	-0.3	8.43	0.432132911	8.43	32594	64.8

Figure 2-12: XIC manager showing pesticide compounds not found in the library.

2.7.1.14 Step three

Peaks with highest intensity are selected. The possible structure is elucidated from the elementary formula and isotopic profile. The elementary formula is loaded on ChemSpider and unlike the target screen and non-target screen approaches where on one possible structure is obtained, many different structures are obtained when identifying compounds that are not in the library. A typical example of elementary composition and isotopic profile pane displayed in Figure 2-13

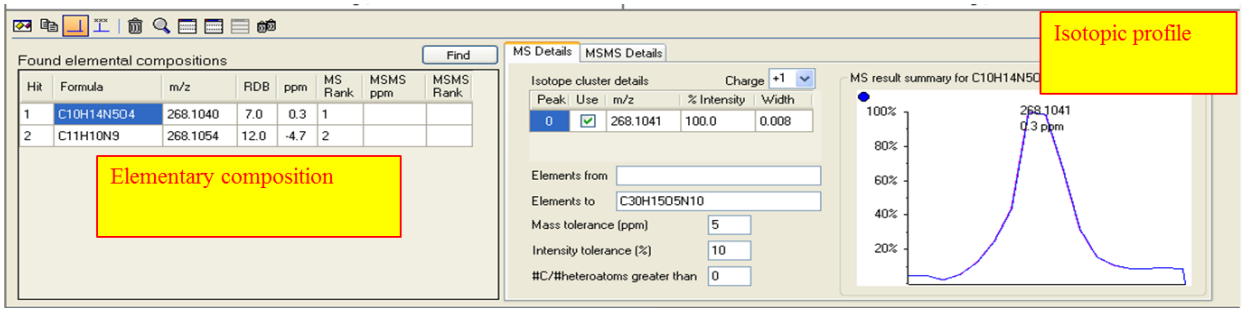


Figure 2-13: Formula finder showing a typical elementary composition pane and isotopic profile pane of a selected peak.

2.7.1.15 Step four

The masses of both the precursor and product ions are linked, to help identify and characterise the compounds. Many structures from the same formula are linked to the MS/MS spectra and fragment pane. The structure with the best fit to the precursor and product ions is the most probable one. A typical example displayed in Figure 2-14

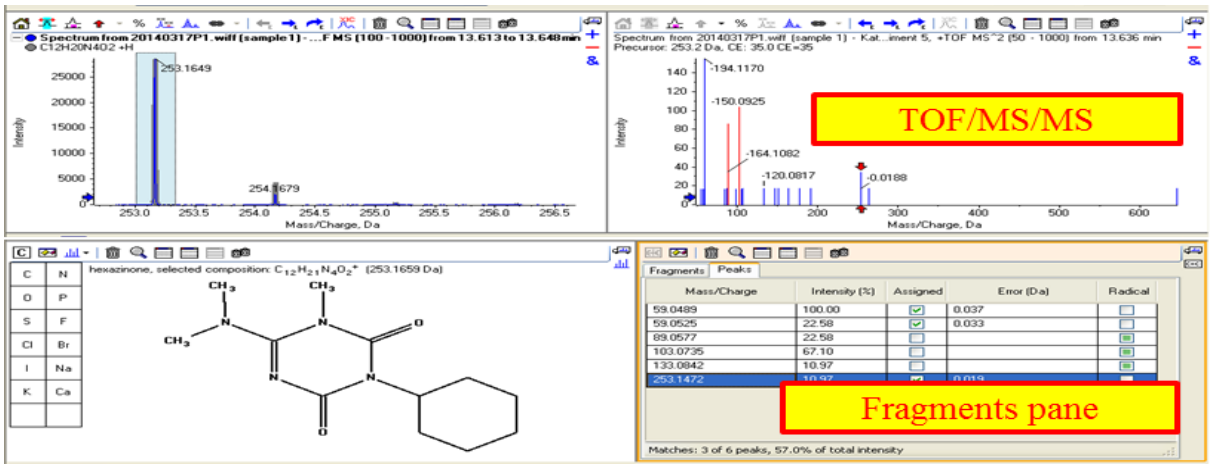


Figure 2-14: Structure elucidation application. The structure is linked to the precursor and product ions.

However the whole breakdown protocol from the raw TIC data to the processed fragment pane confirmation data can be compressed into one pane and a typical example is displayed as shown in Figure 2-15

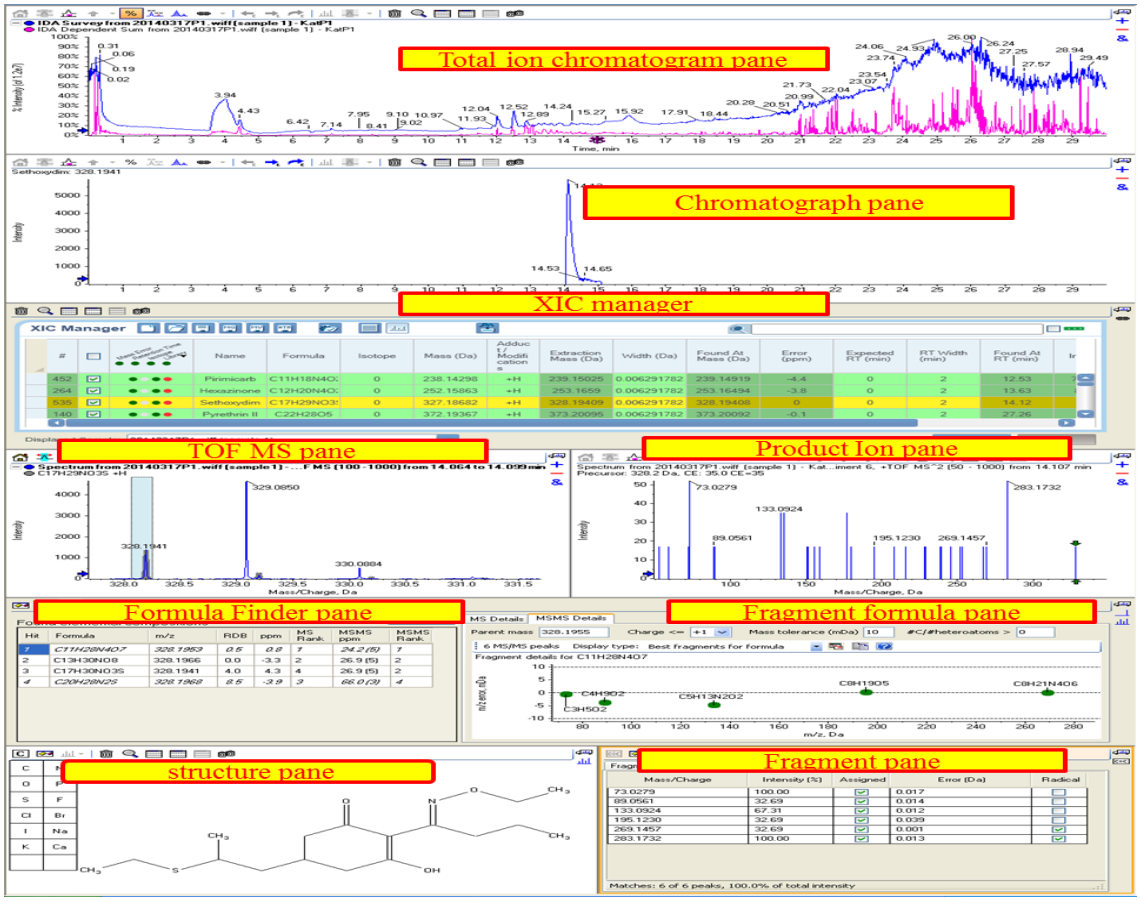


Figure 2-15: Typical example of compressed experimental flow diagram used for the screening of pesticides analytes from river water samples.

2.8 Study area

Kat River Valley's altitude increases from approximately 600 to 1600 m at the top of the escarpment (Shackleton and Shackleton, 2006). Its climate can be described as mild (Magni, 1999). Rainfall is unevenly distributed within the area. It ranges between 400 and 1200 mm, where the least rainfall is received at the confluence with the Great Fish River and the highest, in the mountainous northern region of the catchment (Magni, 1999). The rainfall is relatively high in the mountainous region, but much of the area in the catchment can be regarded as sub-humid to semi-arid. Kat River Valley receives both summer and winter rainfall. Approximately 75% of the mean annual precipitation is received between October/November and February/March, where the highest rainfall figures are recorded in March. The temperatures range from moderately hot summers to cool moderate winters (Monteux, 2001).

The Kat River Valley is characterised by a variety of land uses, ranging from export-oriented citrus farming, commercially oriented rangeland stock farming to small-scale vegetable and crop production and stock farming (McMaster, 2002).

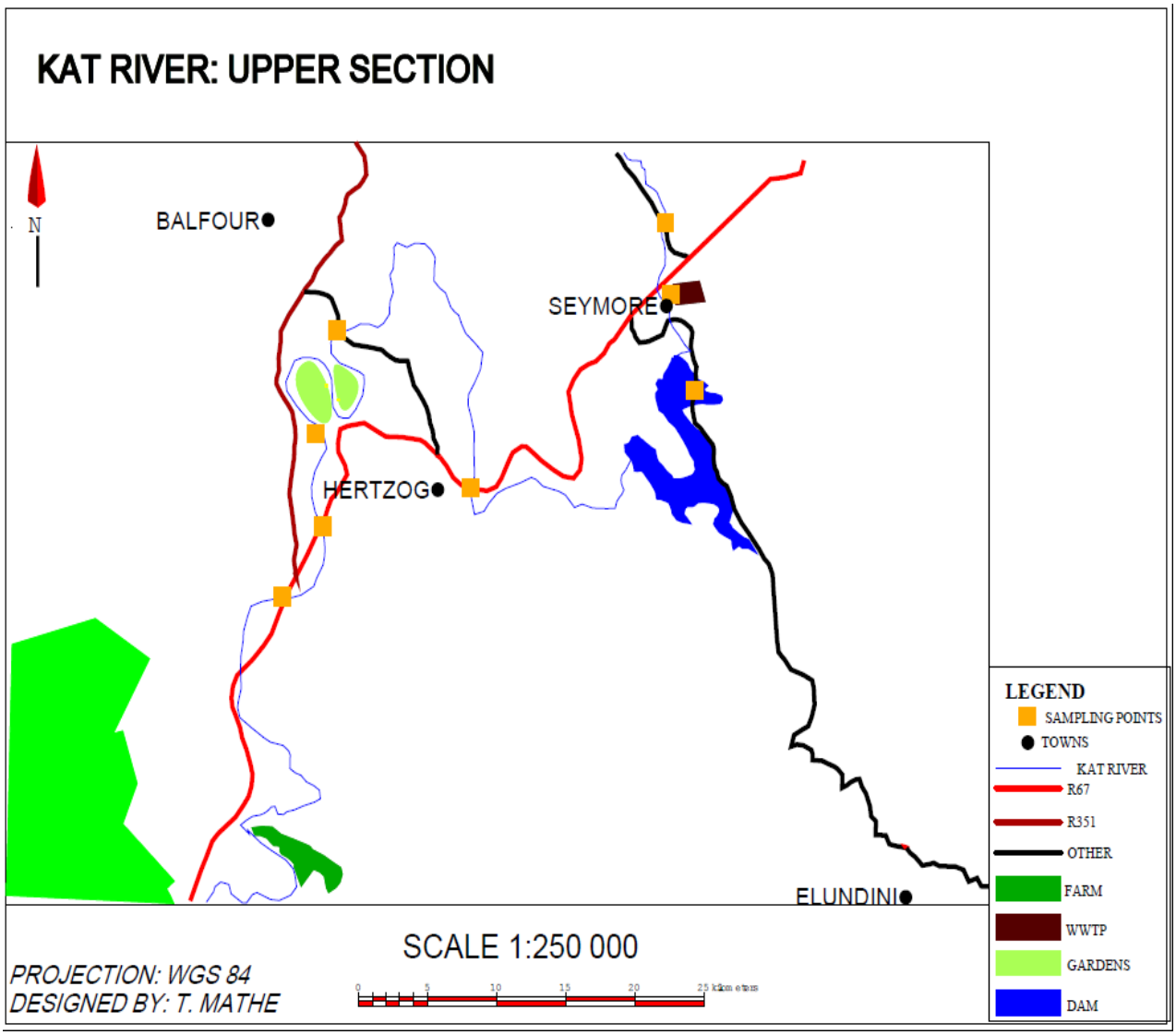


Figure 2-16: Kat River Catchment showing Upper Kat

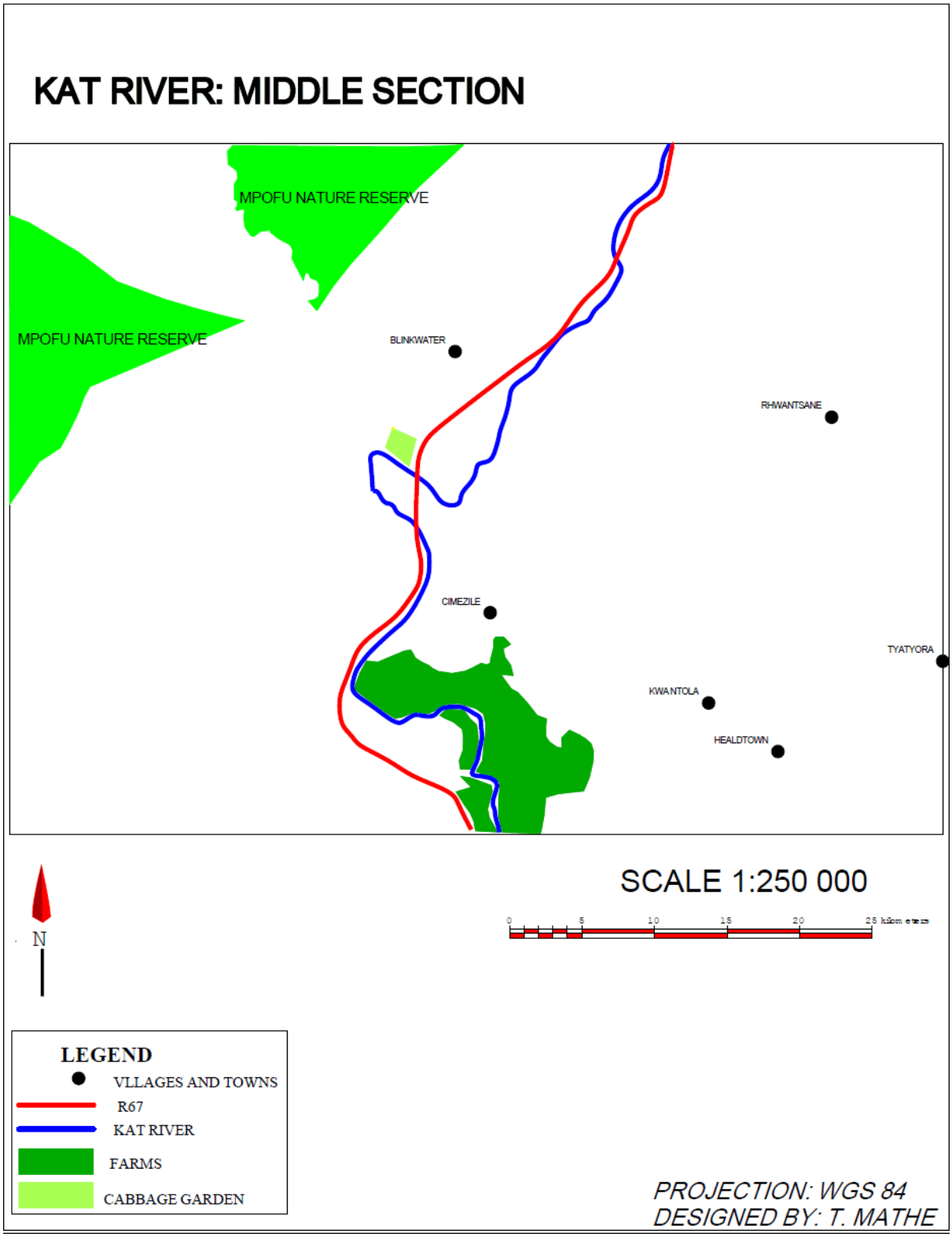


Figure 2-17: Kat River Catchment showing Middle Kat

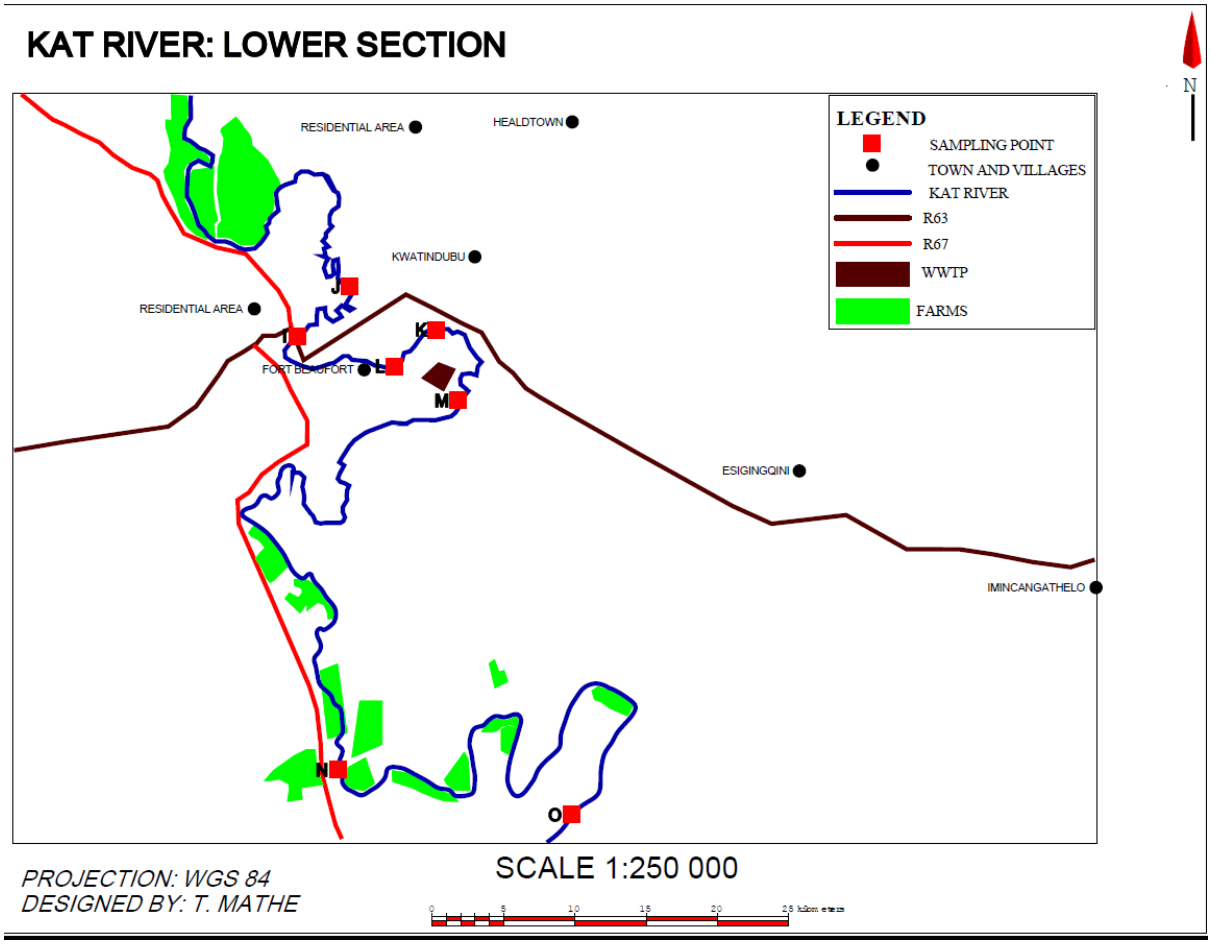


Figure 2-18: Kat River Catchment showing Lower Kat.

Samples were collected wherever Kat River was accessed from R67 or farm roads.Commercial farmers are mainly located in the Middle and Lower Kat, whereas smallholders and emerging farmers mostly practice agriculture in the Upper catchment (Nel, 1998). Vegetable gardening is an important occupation amongst smallholder farmers in the area. Most of these vegetables are grown on fertile plots lying adjacent to rivers and streams. For watering the vegetables, some farmers practice sprinkler irrigation, whereas the farmers who lack irrigation infrastructure practice hand irrigation (Farolfi and Rowntree, 2005). The soil on which most cultivation occurs in the valley is alluvium, which is suitable for agriculture (Smit, 2003). Smit (2003) explains that even though the soil is suitable for agriculture, phosphorous and potassium deficiencies have been identified in the alluvial soil profiles of the Kat River basin. See Figure 2-16 to Figure 2-18 above for Kat river coverage.

2.9 Results and Discussion

The collection times were conducted following the Kat River farmers pesticide application timetable. The following data were obtained from the spray program used by farmers for the period of research. Table 2-2 below shows pesticide application timing, target pest and active ingredient of applied pesticide. This information was important for identifying potential sources of pesticides found in the river samples as well as identifying the pesticides to be used in the targeted approach.

Table 2-2: Kat River Farms pesticide application timing, Target pest and Active ingredient of applied pesticide

APPLICATION TIMING		**REASON**	**PRODUCT DETAILS**	
DATE (GROWTH STAGE)	**SPRAY NUMBER**	**PEST**	**TRADE NAME**	**ACTIVE INGREDIENT**
During late August or early September	**1**	Phytophthora root rot	Phosphite 400 SL	Potassium phosphite 560g/ ℓ (Phosphorous acid equivalent of 400g/ ℓ)
22 September 2013 (50% PLD)	**2**	Bollworm Thrips Red Scale Mealybug	Aquabuff	Organic acids
			Dursban 750 WG	Chlorpyrifos 750g/ kg
			Tebuzole 250 EW	Tebuconazole 250g/ ℓ
			Break-Thru S 240	Polyether-polymethylsiloxane - copolymer 1000g/ ℓ
8 October 2013 (90% PLD)	**3**	Thrips CBS 1	Aquabuff	Organic acids
			Unizeb 800 WP	Mancozeb 800g/ kg

			Klartan 240 EW	Tau-fluvalinate 240g/ ℓ
			Tebuzole 250 EW	Tebuconazole 250g/ ℓ
			Break-Thru S 240	Polyether-polymethylsiloxane - copolymer 1000g/ ℓ
Approximately 6 weeks after previous treatment	**4**	Phytophthora root rot	Phosphite 400 SL	Potassium phosphite 560g/ ℓ (Phophorous acid equivalent of 400g/ ℓ)
01 November 2013	**5**	CBS 2 Thrips Red Scale	Aquabuff	Organic acids
			Unizeb 800 WP	Mancozeb 800g/ kg
			Scalex 100 EC	Pyriproxyfen 100g/ ℓ
			Fungaway 250 SC	Azoxystrobin 250g/ ℓ
			Biomectin 18 EC	Abamectin 18g/ ℓ
			BAC Oil Medium	Med mineral oil
10 December 2013 (42 days after CBS 2)	**6**	CBS 3 Thrips Red Scale	Aquabuff	Organic acids
			Delegate 250 WG	Spinetoram 250g/ kg
			Unizeb 800 WP	Mancozeb 800g/ kg
			Scalex 100 EC	Pyriproxyfen 100g/ ℓ
			Fungaway 250 SC	Azoxystrobin 250g/ ℓ
			BAC Oil Medium	Med mineral oil

14 January 2014 (depending on trap counts)	**7**	Fruit fly	Mercaptothion 500 EC	Mercaptothion 500g/ ℓ
			Humlure RFU	Protein hydrolysate 425g/ ℓ
20 January 2014 (42 days after CBS 3)	**8**	CBS 4 FCM - Cryptex 1	Aquabuff	Organic acids
			Unizeb 800 WP	Mancozeb 800g/ kg
			Cryptex SC	2 X 10^{10} CrleGV/ mℓ
			Biomectin 18 EC	Abamectin 18g/ ℓ
			BAC Oil Medium	Med mineral oil
25 March 2014	**9**	Fruit fly	GF 120 NF	Spinosad 0.24g/ ℓ
21 days before harvest	**10**	FCM - Cryptex 2	Aquabuff	Organic acids
			Cryptex SC	2 X 10^{10} CrleGV/ mℓ
			Break-Thru S 240	Polyether-polymethylsiloxane - copolymer 1000g/ ℓ

The Kat River catchment covers many farms engaging in different types of farming activities and product lines. It was important to get this information as it provides the sense that not all farmers were engaged in the same product lines and hence different pesticide application programmes were used along the Kat River valley. Table 2-3 shows a few of the selected farms and their product line. However there are approximately 28 farms along Kat River.

Table 2-3: Selected farms and main product line

Baddaford	**Eastlands**	Satsuma
Clementines	Clementines	**Riverside**
Lemon	Satsuma	Clementines
Mandarin	**Fernvilla**	Lemon
Navel	Satsuma	Mandarin
Nova	**Martello**	Navel
Satsuma	Clementines	Nova
Vals	Navel	Satsuma
Bryanston	Satsuma	TORROCA
Lemon	**Millbank**	**Winterberg**
Mandarin	Clementines	Navel
Navel	Lemon	Satsuma
Satsuma	Navel	

The Kat River valley has many different plantations for particular product varieties with varying years over which these varieties were planted. Table 2-4 below shows these variations of years over which different varieties were planted, with the oldest variety being planted as late as 1946. This information was important as it revealed that farming has been going on over a long time and potentially different pesticide application programmes were employed before the study period, which may or may not be similar to those used during the study period. These application programmes may or may not have contained pesticides which today are banned from use. This information played an important role during the analysis of pesticides, as potential persistent pesticides which may no longer be in use today, could be identified as in the river water.

Table 2-4: Variations of years over which different varieties were planted on different farms

FARM	VARIETY	YEAR PLANTED
Baddaford	Vals	1992
Baddaford	Vals	1992
Baddaford	Clementines	1987
Baddaford	Clementines	1987
Baddaford	Clementines	1987
Baddaford	Clementines	1987
Baddaford	Lemon	1972
Baddaford	Lemon	1993
Baddaford	Mandarin	2012
Baddaford	Mandarin	2008
Baddaford	Mandarin	2009
Baddaford	Mandarin	2012
Baddaford	Mandarin	2001
Baddaford	Mandarin	2008
Baddaford	Vals	2012
Baddaford	Mandarin	2010
Baddaford	Mandarin	2010
Baddaford	Vals	2012
Baddaford	Mandarin	2003
Baddaford	Mandarin	2008

Baddaford	Navel	1985
Baddaford	Navel	1950
Baddaford	Navel	1996
Baddaford	Navel	1987
Baddaford	Navel	1950
Baddaford	Navel	1950
Baddaford	Navel	1990
Baddaford	Navel	2008
Baddaford	Navel	2004
Baddaford	Navel	1990
Baddaford	Navel	2004
Baddaford	Navel	1990
Baddaford	Nova	2012
Baddaford	Satsuma	1978
Baddaford	Vals	1992
Baddaford	Vals	1992
Reileyvale	Clementines	1988
Reileyvale	Clementines	1988
Reileyvale	Clementines	1988
Reileyvale	Satsuma	1989
Reileyvale	Satsuma	1989
Reileyvale	Satsuma	1989

Reileyvale	Navel	2008
Reileyvale	Navel	2008
Reileyvale	Navel	2008
Reileyvale	Navel	2008
Reileyvale	Vals	2008
Reileyvale	Vals	2008
Reileyvale	Vals	2008
Reileyvale	Vals	2008
Orange Grange	Navel	2009
Bryanston	Lemon	1982
Bryanston	Mandarin	2002
Bryanston	Navel	2002
Bryanston	Navel	1982
Bryanston	Satsuma	1990
Bryanston	Satsuma	2004
Orange Grange	Navel	2009
Orange Grange	Nova	2009
Orange Grange	Poms	2012
Orange Grange	Poms	2012
Eastlands	Clementines	2005
Eastlands	Clementines	2005
Eastlands	Clementines	2005

Eastlands	Clementines	1989
Eastlands	Clementines	1988
Eastlands	Satsuma	1992
Eastlands	Clementines	1992
Eastlands	Satsuma	1990
Eden	Navel	1988
Eden	Navel	1988
Eden	Navel	1978
Eden	Poms	2012
Fernvilla	Satsuma	1992
Fernvilla	Satsuma	1991
Fernvilla	Satsuma	1994
Fernvilla	Satsuma	1990
Fernvilla	Satsuma	1989
Fernvilla	Satsuma	1990
Fernvilla	Satsuma	1990
Fernvilla	Satsuma	1990
Hopefield	hibush	2012
Hopefield	Poms	2012
Hopefield	Poms	2012
Hopefield	Poms	2012
Jericho	Navel	1987

Jericho	Navel	1986
Jericho	Navel	1996
Jericho	Navel	1986
Jericho	Navel	1985
Jericho	Satsuma	1986
Jericho	Satsuma	1987
Jericho	Satsuma	1985
Jerusalem	Navel	1986
Jerusalem	Navel	1987
Jerusalem	Navel	2012
Jerusalem	Poms	2012
Jerusalem	Poms	2012
Krilla	Poms	2012
Krilla	Poms	2012
Krilla	Poms	2012
Krilla	Poms	2012
Krilla	Poms	2012
Krilla	Poms	2012
Krilla	Poms	2012
Krilla	Poms	2012
Krilla	Poms	2012
Krilla	Poms	2012
Krilla	Poms	2012

Krilla	Poms	2012
Krilla	Poms	2012
Orange Grange	Poms	2012
Orange Grange	Poms	2012
Orange Grange	Poms	2012
Orange Grange	Poms	2012
Orange Grange	Satsuma	2012
Oakdene	Navel	2008
Oakdene	Navel	2008
Oakdene	Navel	2008
Oakdene	Navel	2008
Oakdene	Navel	2008
Oakdene	Navel	2008
Oakdene	Navel	2008
Oakdene	Clementines	2011
Oakdene	Mandarin	2011
Oakdene	Nova	2011
Oakdene	Navel	2012
Oakdene	Navel	2012
Oakdene	Navel	2012
Naudeshoek	Navel	1974
Naudeshoek	Navel	1986

Naudeshoek	Clementines	1989
Naudeshoek	Clementines	1989
Naudeshoek	Navel	1997
Naudeshoek	Navel	1997
Naudeshoek	Navel	1997
Naudeshoek	Clementines	1989
Naudeshoek	Clementines	1989
Naudeshoek	Navel	1989
Naudeshoek	Navel	1998
Naudeshoek	Navel	1998
Naudeshoek	Navel	1998
Naudeshoek	Navel	2008
Naudeshoek	Navel	2008
Naudeshoek	Navel	2008
Naudeshoek	Navel	2008
Naudeshoek	Navel	2008
Naudeshoek	Navel	2008
Naudeshoek	Navel	2008
Naudeshoek	Navel	2008
Naudeshoek	Navel	2008
Naudeshoek	Navel	2008
Naudeshoek	Vals	2008
Naudeshoek	Vals	2008

Naudeshoek	Vals	2008
Naudeshoek	Lemon	2012
Martello	Clementines	1988
Martello	Clementines	1987
Martello	Navel	1986
Martello	Satsuma	1990
Martello	Satsuma	1988
Millbank	Clementines	1993
Millbank	Grapefruit	1996
Millbank	Lemon	1985
Millbank	Lemon	2011
Millbank	Navel	2012
Millbank	Navel	1996
Millbank	Navel	1982
Millbank	Navel	1999
Millbank	Navel	1998
Millbank	Navel	1986
Millbank	Navel	1995
Millbank	Satsuma	1992
Millbank	Satsuma	2001
Millbank	Satsuma	1991
Millbank	Satsuma	1991

Lovers Retreat	Navel	2009
Lovers Retreat	Navel	2009
Lovers Retreat	Poms	2009
Lovers Retreat	Poms	2009
Lovers Retreat	Poms	2009
Lovers Retreat	Poms	2009
Lovers Retreat	Poms	2009
Lovers Retreat	Poms	2009
Lovers Retreat	Satsuma	2010
Lovers Retreat	Satsuma	2010
Lidell	Navel	1986
Lidell	Navel	1986
Lidell	Navel	1986
Lidell	Navel	1989
Lidell	Navel	1989
Ndakana	hibush	2012
Ndakana	Poms	2012
Lidell	Navel	1989
Lidell	Navel	1989
Lidell	Navel	2008
Lidell	Navel	2008
Lidell	Navel	2008

Lidell	Navel	2011
Lidell	Navel	2010
Lidell	Navel	1986
Lidell	Poms	2011
Lidell	Poms	2012
Leta's Farm	Navel	1986
Leta's Farm	Navel	2009
Leta's Farm	Navel	2009
Leta's Farm	Navel	2009
Leta's Farm	Navel	2009
Leta's Farm	Navel	2009
Leta's Farm	Nova	2009
Leta's Farm	Nova	2009
Leta's Farm	Nova	2009
Leta's Farm	Lemon	2011
Leta's Farm	Navel	2011
Leta's Farm	Navel	2012
Cottage	Navel	1946
Cottage	Navel	1946
Cottage	Clementines	1988
Cottage	Clementines	1994
Cottage	Navel	1995

Cottage	Navel	2008
Cottage	Navel	2008
Cottage	Satsuma	2009
Cottage	Clementines	2012
Cottage	Lemon	2012
Cottage	Satsuma	2012
Battlesden CPA	Nova	2011
Battlesden	Poms	2011
Picardy	hibush	2012
Picardy	Poms	2012
Battlesden CPA	Navel	2012
Battlesden	Clementines	2012
Battlesden CPA	hibush	2012
Battlesden CPA	Navel	2012
Battlesden CPA	Navel	2012
Battlesden CPA	Navel	2012
Battlesden CPA	Poms	2012
Battlesden	Navel	1980
Battlesden	Navel	1986
Battlesden	Clementines	2012
Battlesden CPA	Poms	2012
Battlesden	Satsuma	2012

Riverside	Lemon	2012
Riverside	Lemon	2011
Riverside	Lemon	1998
Riverside	Lemon	1998
Riverside	Mandarin	2012
Riverside	Mandarin	2012
Riverside	Mandarin	2011
Riverside	Mandarin	2012
Riverside	Mandarin	2012
Riverside	Mandarin	2011
Riverside	Mandarin	2012
Riverside	Navel	1985
Riverside	Navel	2006
Riverside	Navel	2003
Riverside	Clementines	2012
Riverside	Navel	1991
Riverside	Navel	1991
Riverside	Lemon	1969
Riverside	Navel	1991
Riverside	Navel	1999
Riverside	Navel	1991
Riverside	Mandarin	2008

Riverside	Navel	2004
Riverside	Navel	1987
Riverside	Lemon	1969
Riverside	Navel	1991
Riverside	Navel	2003
Riverside	Navel	1970
Riverside	Navel	2004
Riverside	Navel	2000
Riverside	Nova	2000
Riverside	Nova	2002
Riverside	Satsuma	1982
Riverside	Satsuma	1978
Riverside	Satsuma	1976
Riverside	Satsuma	1982
Riverside	Satsuma	1978
Riverside	Satsuma	2003
Riverside	Satsuma	1982
Riverside	satsuma	2011
Riverside	torroca	2004
Thorndale Comm.	hibush	2012
Thorndale Comm.	hibush	2012
Thorndale Comm.	Poms	2012

Thorndale Comm.	Poms	2012
Thorndale Comm.	Poms	2012
Top Kat	Navel	1995
Top Kat	Navel	1995
Top Kat	Navel	1995
Top Kat	Navel	2009
Top Kat	Poms	2012
Top Kat	Poms	2012
Top Kat	Poms	2012
Winterberg	Navel	1985
Winterberg	Navel	1970
Winterberg	Navel	2001
Winterberg	Navel	2004
Winterberg	Satsuma	2002
Winterberg	Satsuma	2002

Target screen pesticide analysis

Pesticides form part of the small molecules which requires sophisticated equipment to identify and quantify them. The new LC/MS/MS technique with electrospray ionization is a technique of choice best suited for targeted identification of pesticides. The 5600 AB SCIEX Triple TOF has high selectivity and sensitivity and is able to screen for, quantify, and identify large panels of analytes across different compound classes in a single scan which has made it invaluable for small molecule analysis throughout the world.

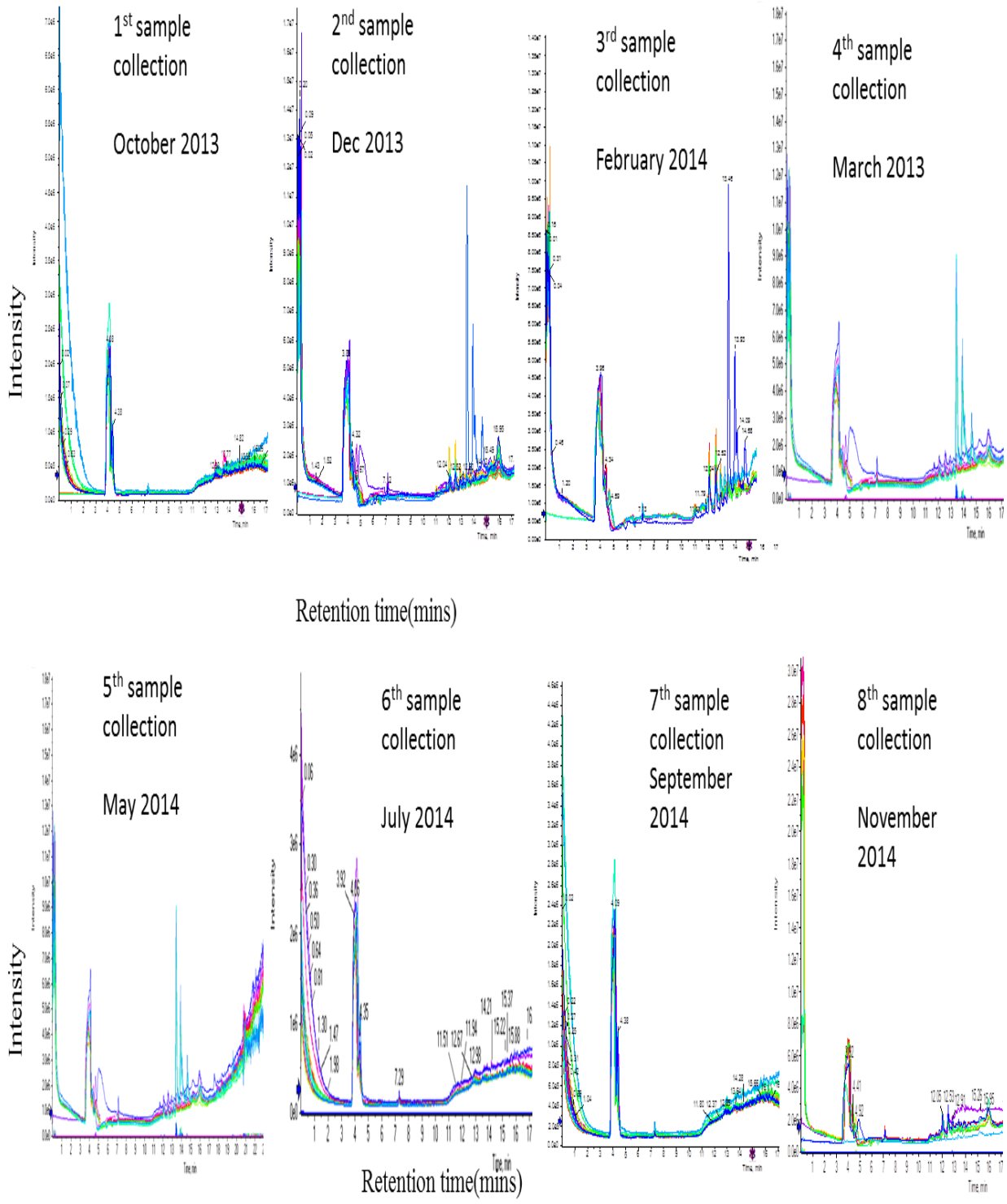


Figure 2-19: Total Ion Chromatography (TIC) obtained by combining all mass spectra from 15 samples collected during the same day, from various points along the Kat River.

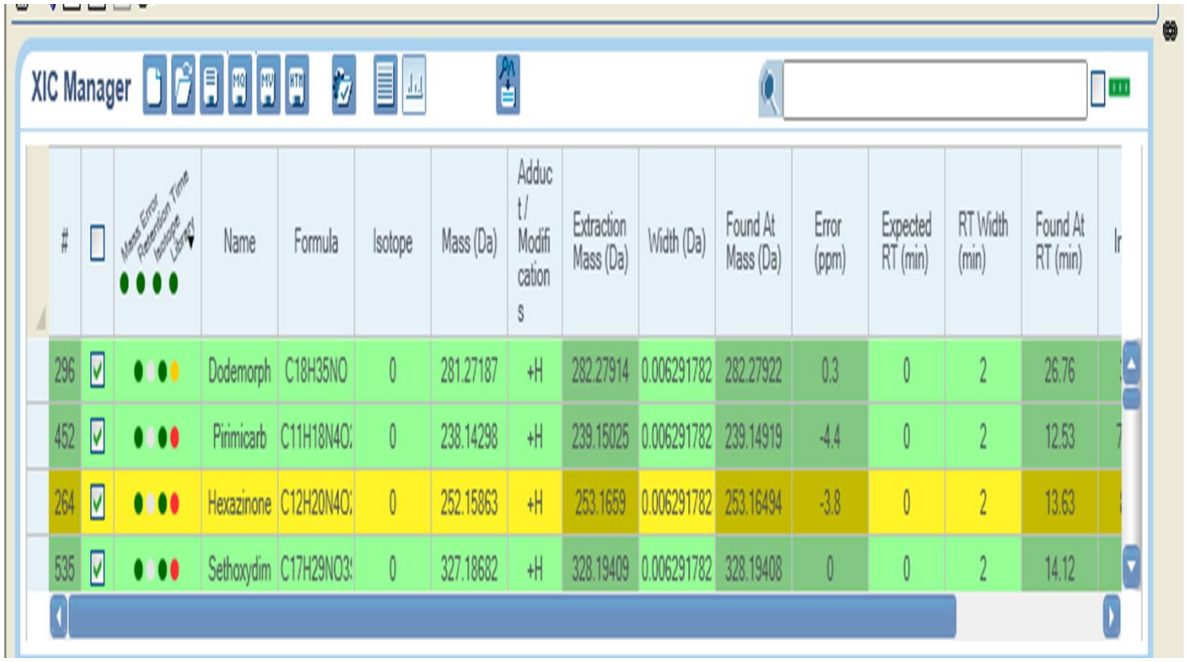


Figure 2-20: Typical example of XIC manager obtained by running the TIC against the pesticide library using the target screen approach.

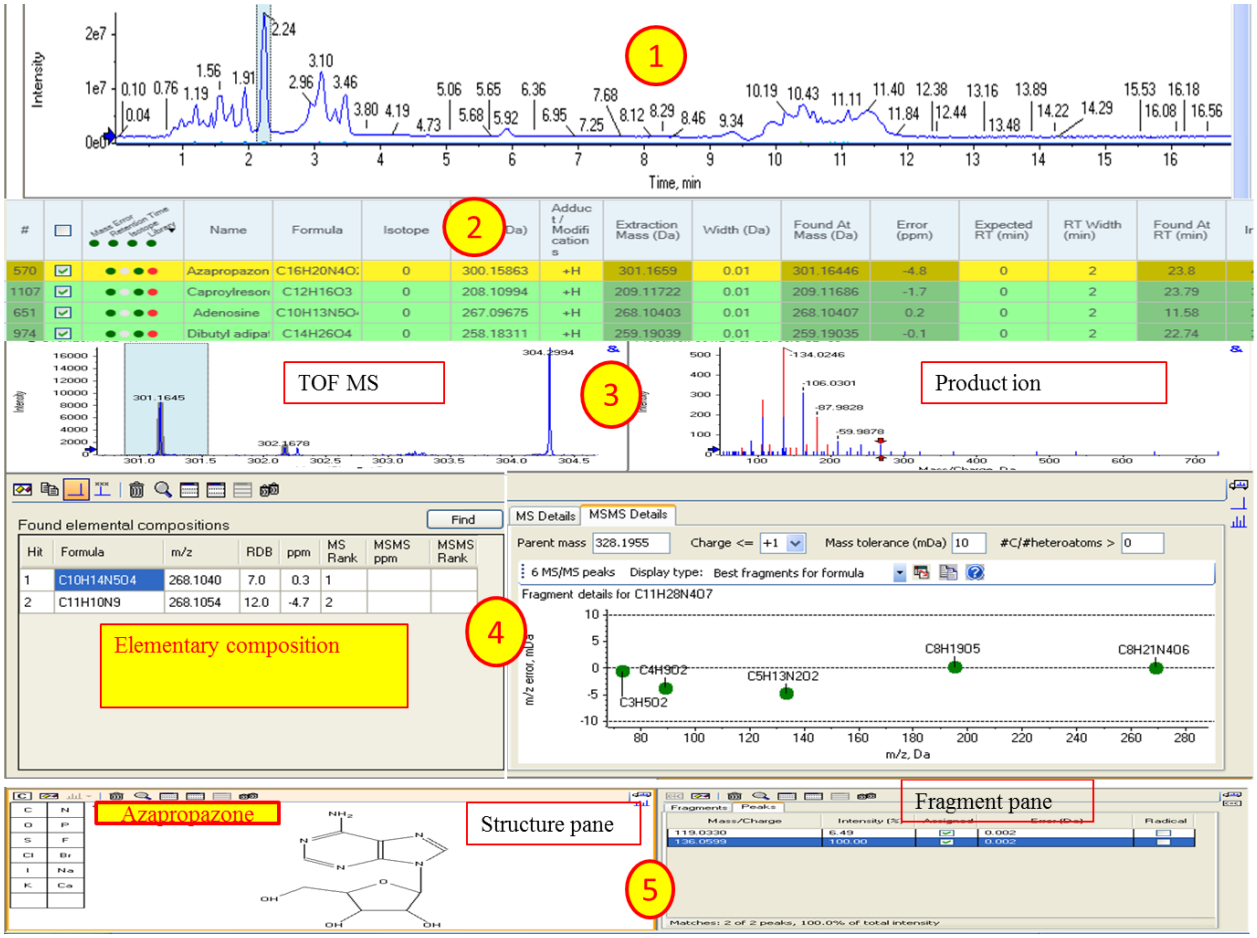


Figure 2-21: Typical example compressed experimental flow diagram for Azapropazone pesticide identified using the target screen approach.

Table 2-5: Typical example of pesticides identified using the target screen approach

Index	CompoundName	Formula	Mass	Mass error	Retention time	Purity Score	Intensity
POINT C							
	Spiroxamine	C18H35NO2	297.2667797	7.098864552	12.12456987	98	1418.059281
	Prometryne	C10H19N5S	241.1361176	7.220662214	13.00768936	76	1539
	Pyriproxyfen	C20H19NO3	321.1364937	7.342527349	13.67543785	100	2825.483392
Point E							
	Spiroxamine	C18H35NO2	297.2667797	8.6088784	11.35325286	100	1652.059281
	Prometryne	C10H19N5S	241.1361176	7.894573625	13.46076894	56	1234
	Pyriproxyfen	C20H19NO3	321.1364937	8.000736251	13.12345576	100	2890.938477
Point F							
	Azoxystrobin	C22H17N3O5	403.1168209	12.8529447	13.75685343	100	13245.94858
	Pyriproxyfen	C20H19NO3	321.1364937	7.572615378	14.09488463	100	3045.784598
	Atrazine	C8H14ClN5	215.0937733	3.910315001	11.68686361	65	1468.577209
	Desmethyl-pirimicarb	C10H16N4O2	224.127326	6.388477474	13.10240049	78	1367.577913
	Sethoxydim	C17H29NO3S	327.1868159	0.864063881	23.68293551	45	1072.086193
	Spirodiclofen	C21H24Cl2O4	410.105165	0.356516701	25.60876484	86	3804.744001
	Spinetoram A	C42H69NO10	747.4921482	0.311299147	20.22485101	86	1680.104717
Point G							
	Pyriproxyfen	C20H19NO3	321.1364937	7.377289105	13.46076894	95	3287.987636
Point H							
	Spirodiclofen	C21H24Cl2O4	410.105165	0.356516701	25.60876484	100	4087.293485
	Pyriproxyfen	C20H19NO3	321.1364937	7.220662214	13.67898297	100	4098.098747
	Spinetoram B	C43H69NO10	759.4921482	0.291631534	21.5440663	98	1358.120677
	Imidacloprid	C9H10ClN5O2	255.0523024	2.098765342	16.69876353	100	4261.789655
Point I							
	Dodemorph	C18H35NO	281.271865	0.510035155	15.77950791	100	121640.247
	Pirimicarb	C11H18N4O2	238.142976	-0.688856072	8.61296887	92	33821.65235
	Pyriproxyfen	C20H19NO3	321.1364937	8.000005637	13.48762549	98	3908.097365
Point J							
	Buprofezin	C16H23N3OS	305.1561844	-2.929023994	8.787435781	94	29346.37179
	Spinetoram B	C43H69NO10	759.4921482	0.346315345	21.12345663	98	1234.164723
Point K							
	Imidacloprid	C9H10ClN5O2	255.0523024	0.895324199	15.98764528	100	43551.34587
	Spirodiclofen	C21H24Cl2O4	410.105165	0.356516701	25.60876484	100	7562.736354
Point M							
	Tetramethrin	Tetramethrin	Tetramethrin	7.751498835	20.57016911	34	18128.90897
	Avermectin B1a	Avermectin B1a	Avermectin B	6.763576489	29.7342362	76	5912.67648
Point N							
	Azoxystrobin	C22H17N3O5	403.1168209	12.22837562	13.74746586	89	1987.839279
	Imidacloprid	C9H10ClN5O2	255.0523024	1.267890365	16.13452679	76	23456.23458
	Atrazine	C8H14ClN5	215.0937733	4.910315001	11.35467865	44	1124.545839
Point O							
	Imidacloprid	C9H10ClN5O2	255.0523024	0.621933695	16.60684531	56	12453.13407
	Atrazine	C8H14ClN5	215.0937733	3.810315001	11.23678905	56	1315.577209
	Carbendazim	C9H9N3O2	191.0694767	0.862147002	26.65864889	98	1695.831824

Graph 2-1: A graph of Spiroxamine detected at multiple sample sites for various sample collection days.

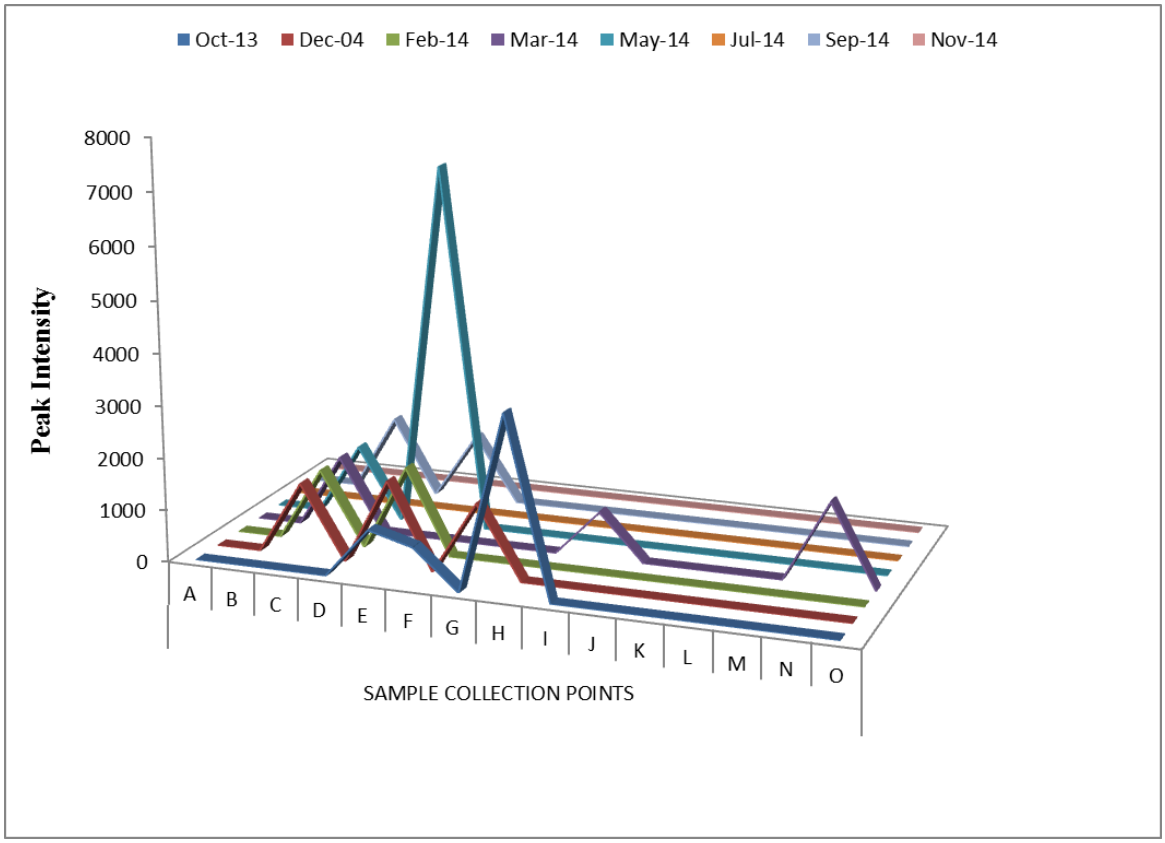


Table 2-6: A summary of all the pesticides identified in the Kat River during period November 2013-November 2014

	Oct 2013 sample collection															Dec 2013 sample collection														Feb 2013 sample collection														March 2014 Sample collection													
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	A	C	E	F	G	H	I	J	K	M	N	O	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Acetochlor																																																							
Atrazine						✓													✓							✓							✓							✓	✓					✓										
Avermectin B1a																									✓														✓																	
Azoxystrobin														✓					✓							✓							✓							✓				✓	✓			✓								
Buprofezin																						✓																✓								✓										
Carbendazim															✓																																											
Cypermethrin																																																								
Desmethyl-pirimicarb																			✓															✓																						
Imidacloprid											✓									✓		✓			✓	✓							✓							✓	✓					✓	✓			✓	✓				
Imidacloprid-Olefin																																																						
Pirimicarb																				✓																																						
Prometryne						✓											✓	✓													✓		✓													✓											
Pyriproxyfen							✓											✓	✓		✓	✓									✓		✓	✓	✓	✓	✓							✓	✓	✓		✓						
Sethoxydim																			✓															✓														✓	✓		✓					✓
Spirodiclofen																			✓				✓											✓		✓																				✓
Spiroxamine																	✓	✓		✓											✓		✓													✓				✓				✓	
Thiabendazole																																																						
Spinetoram A						✓																												✓																						
Spinetoram B																				✓	✓														✓	✓																			
Tetrametrin																							✓																✓																✓	✓
Acetochlor																																																					

	May 2014 Sample collection															July 2014 Sample collection														September 2014 Sample collection														November 2014 Sample collection													
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Acetochlor																																																							
Atrazine						✓							✓															✓															✓	✓													✓	✓
Avermectin B1a												✓															✓															✓																
Azoxystrobin						✓							✓								✓														✓																			✓		
Buprofezin										✓															✓																																
Carbendazim														✓																																												✓
Cypermethrin																																																								
Desmethyl-pirimicarb																																																								
Imidacloprid											✓		✓									✓			✓													✓		✓			✓								✓		✓				
Imidacloprid-Olefin																																																							
Pirimicarb									✓																	✓																																
Prometryne					✓													✓		✓	✓																										✓		✓										
Pyriproxyfen							✓		✓												✓		✓	✓																✓		✓																
Sethoxydim																																																							
Spirodiclofen						✓		✓			✓															✓												✓																	✓			
Spiroxamine			✓		✓																											✓		✓																					
Thiabendazole																																																						
Spinetoram A						✓															✓														✓																					
Spinetoram B									✓										✓	✓					✓																						✓										
Tetrametrin																												✓																										✓	

The results obtained from the analysis of pesticides using the targeted screen approach shows that there was high concentration levels of pesticides collected in the months December (2013), February (2014), March (2014), May (2014) and July (2014) compared to those collected from October (2013), September (2014) and November (2014) ,see Figure 2-19.The difference in concentrations levels is due to the differences in precipitation levels with the former having the highest concentration levels compared to the later hence more pesticides washed from the farms into the river.

The detection of pesticide compounds using the targeted screen approach was achieved by screening the TICs (see Figure 2-19) against the pesticide library with the aid of the XIC manager (see Figure 2-20). The detected pesticide compounds were confirmed by using different software software's within the PeakView software in an experimental flow diagram (see Figure 2-21 and Annex 3 for other results of pesticides detected using the targeted approach)

The results in Table 2-5 and Table 2-6 show that some pesticides were detected at one point and were not detected at successive point immediately after the point pf detection. Typical examples of this interpretation are pesticides Spiroxamine and Prometryne detected at sample C but not detected at point. They were however detected at point E which is further downstream. This means that the sources of Spiroxamine and Prometryne are the farms at points C and E and these farms are using the same spray regime. However these pesticides degraded rapidly in the river.

However Pyriproxyfen was detected at point C and points E, F, G, H and I with concentration increasing at F, G, and H but decreasing concentration at I. This means that the sources of pyriproxyfen are farms around points C, E, F, G, and H. The farms around these points are all having either lemon or orange orchards and therefore might be using the same spray programme resulting in increased intensities of Pyriproxyfen downstream of Kat River.

Spirodiclofen was detected at point F and successive points downstream at increasing concentrations. The sources of Spirodiclofen are farms around the points of detection and these farms are using the same spray regime. However Spinetoram was detected at point F and successive points downstream but at decreasing concentrations. This means that the source of Spinetoram is farms around point F. This also means that Spinetoram is persistent (degrades slowly) and contaminates the Kat River downstream.

From the above interpretation, results have shown there is downstream water contamination in Kat River as some pesticides degrade slowly and hence persistent in the environment. The fate of these pesticides is obviously affecting downstream agricultural activities and also communities living found on/near the Kat River banks.

Non-target screening of pesticides in Kat River water analysis

Non-target analysis is the screening of pesticide compounds retrospectively from within the pesticide library.

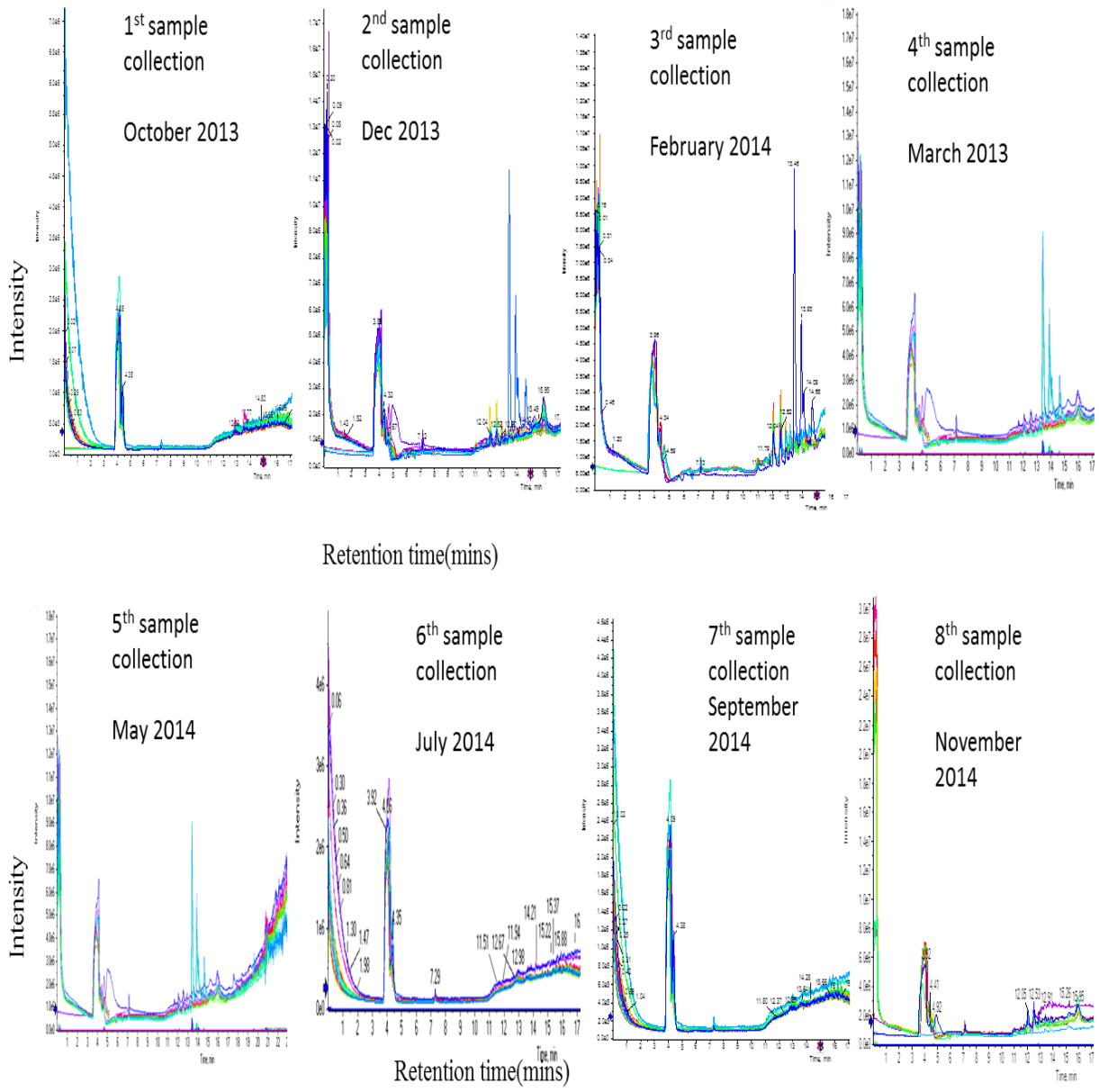
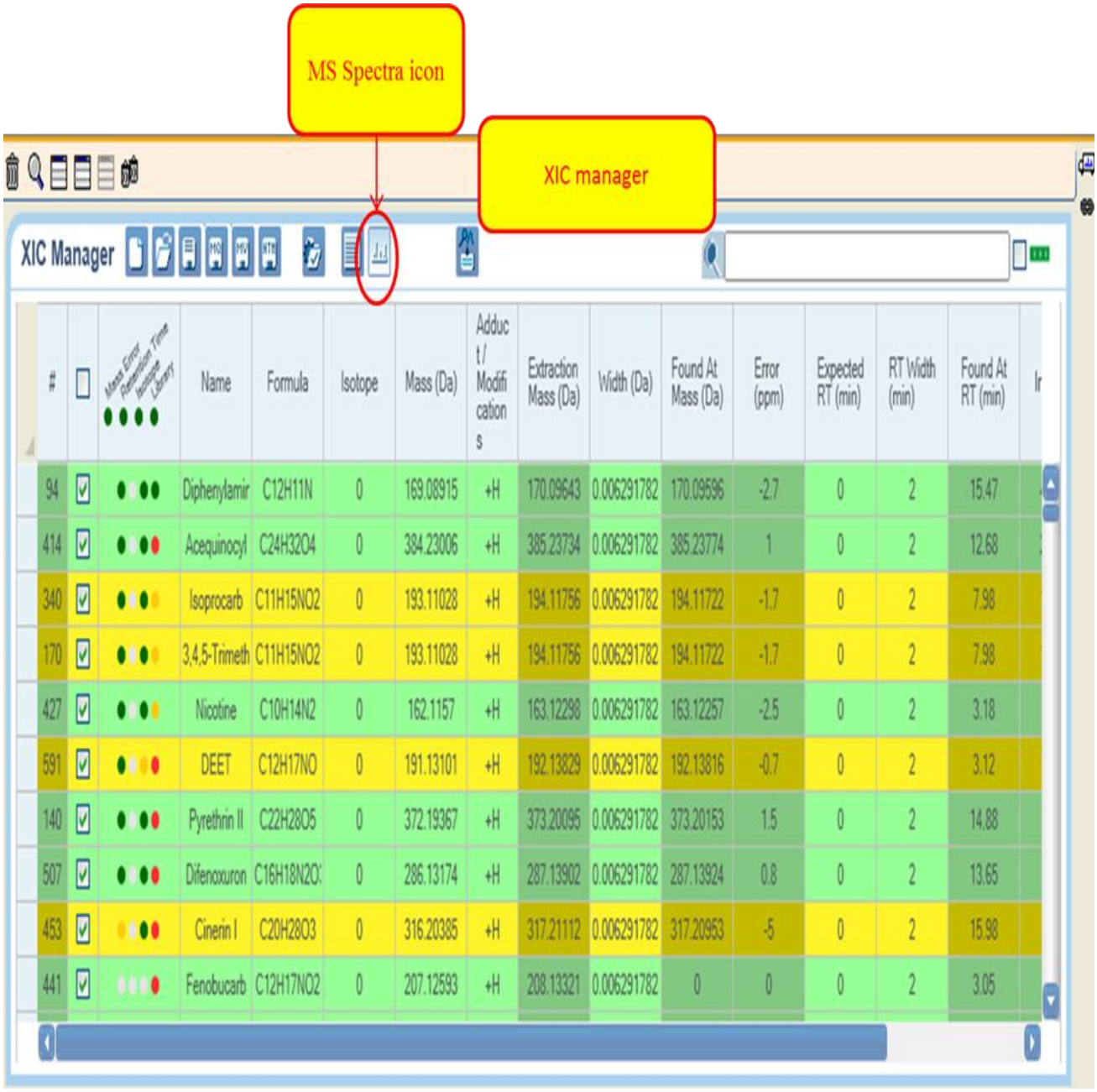


Figure 2-22:Total Ion Chromatography (TIC) obtained by combining all mass spectra from 15 samples collected during the same day, from various points along the Kat River.



#	Name	Formula	Isotope	Mass (Da)	Adduct / Modifications	Extraction Mass (Da)	Width (Da)	Found At Mass (Da)	Error (ppm)	Expected RT (min)	RT Width (min)	Found At RT (min)
94	Diphenylamir	C12H11N	0	169.08915	+H	170.09643	0.006291782	170.09596	-2.7	0	2	15.47
414	Acequinocyl	C24H32O4	0	384.23006	+H	385.23734	0.006291782	385.23774	1	0	2	12.68
340	Isoprocarb	C11H15NO2	0	193.11028	+H	194.11756	0.006291782	194.11722	-1.7	0	2	7.98
170	3,4,5-Trimeth	C11H15NO2	0	193.11028	+H	194.11756	0.006291782	194.11722	-1.7	0	2	7.98
427	Nicotine	C10H14N2	0	162.1157	+H	163.12298	0.006291782	163.12257	-2.5	0	2	3.18
591	DEET	C12H17NO	0	191.13101	+H	192.13829	0.006291782	192.13816	-0.7	0	2	3.12
140	Pyrethrin II	C22H28O5	0	372.19367	+H	373.20095	0.006291782	373.20153	1.5	0	2	14.88
507	Difenoxuron	C16H18N2O3	0	286.13174	+H	287.13902	0.006291782	287.13924	0.8	0	2	13.65
453	Cinerin I	C20H28O3	0	316.20385	+H	317.21112	0.006291782	317.20953	-5	0	2	15.98
441	Fenobucarb	C12H17NO2	0	207.12593	+H	208.13321	0.006291782	0	0	0	2	3.05

Figure 2-23: Typical example of XIC manager obtained by running the TIC against the pesticide library using the non-target screen approach.

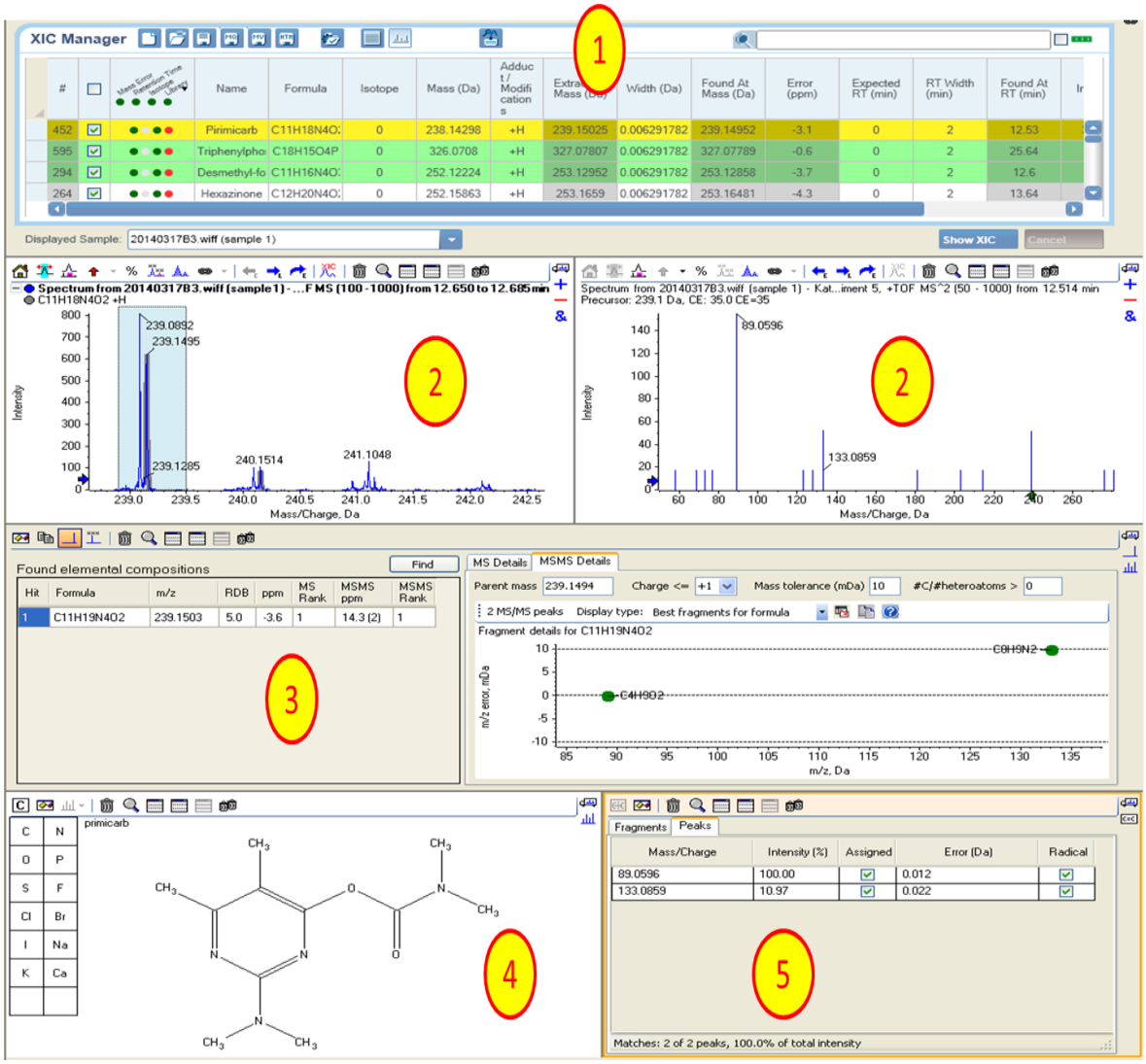


Figure 2-24: Typical experimental flow diagram for the identification of the pesticides using the non-target screen approach :(1) Shows results of non-targeted results displayed in the XIC pane. (2) Shows the TOF-MS pane and Product ion pane obtained by clicking any of the identified pesticides in the XIC pane. (3) Shows the formula finder pane and MS/MS details pane. (4) Shows the structure of the identified pesticide. (5) Show the fragments pane.

Table 2-7: Typical example of non-target pesticide compounds identified in the Kat River water samples based on MS/MS library searching obtained from February 2014 sample collection.

Index	CompoundName	Formula	Mass	Mass error	Retention time	LibraryScore	Intensity
Point B	Point B						
	3,4,5-Trimethacarb	C11H15NO2	193.1102789	0.164148511	7.890574611	96	15054.38388
	Isoprocarb	C11H15NO2	193.1102789	4.164148511	7.890574611	34	1453.096857
	Hexazinone	C12H20N4O2	252.1586261	0	9.756322	100	70094.94886
Point C							
	DEET	C12H17NO	191.1310143	2.931235739	8.48973514	80	4781.41244
	Hexazinone	C12H20N4O2	252.1586261	0	9.756322	100	70094.94886
	Atrazine-desethyl	C6H10N5Cl	187.0624732	7.129052174	15.9857563	80	2088.512869
Point D							
	Diphenylamine	C12H11N	169.0891494	2.776578937	15.52397201	89	3284.965892
	Promecarb	C12H17NO2	207.125929	3.59506607	8.229715154	67	3097.683994
	Fenobucarb	C12H17NO2	207.125929	3.59506607	8.229715154	100	3097.683994
Point E							
	Isoprocarb	C11H15NO2	193.1102789	5.95876222	7.948475653	56	1654.485767
	Hexazinone	C12H20N4O2	252.1586261	-0.599479535	8.99685763	89	5978.069687
Point F							
	DEET	C12H17NO	191.1310143	2.103948575	9.049857463	98	4781.41244
	Isoprocarb	C11H15NO2	193.1102789	4.968757322	6.097865743	76	1875.069698
	Hexazinone	C12H20N4O2	252.1586261	-0.209686774	9.564849922	98	5678.07897
Point G							
	Cinerin I	C20H28O3	316.203845	1.986563	13.9755633	100	2345.906587
	Warfarin	C19H16O4	308.1048592	0.546756868	13.04958575	56	18765.05967
	Imazamethabenz-methyl	C16H20N2O3	288.1473927	0	13.89252472	58	1218.426458
Point H							
	Atrazine-desethyl	C6H10N5Cl	187.0624732	8.059568675	15.09585765	45	2088.512869
	Atrazine-desethyl-2-hydroxy	C6H11ON5	169.0963601	4.826243612	9.499750143	92	1621.238154
Point I							
	DEET	C12H17NO	191.1310143	0.857937763	9.094585733	100	5673.096868
	Cyromazine	C6H10N6	166.0966944	1.942442659	15.15047433	78	4748.517567
	Isoprocarb	C11H15NO2	193.1102789	2.98675764	7.96048332	85	2839.986746
Point J							
	Cinerin I	C20H28O3	316.203845	2.09585743	14.756333	85	1387.09875
	Isoprocarb	C11H15NO2	193.1102789	0.9857463	6.0978844	98	4867.098788
	Hexazinone	C12H20N4O2	252.1586261	-0.65748333	9.84746522	75	5467.75884
Point K							
	Diphenylamine	C12H11N	169.0891494	0.8957554	15.94857563	100	4567.097887
	Promecarb	C12H17NO2	207.125929	0.85763333	9.857576333	100	5093.458576
	Isoprocarb	C11H15NO2	193.1102789	0.67589933	7.568474654	98	4984.069987
Point L							
	Promecarb	C12H17NO2	207.125929	2.0948755	9.0958567	87	4059.968686
	Fenobucarb	C12H17NO2	207.125929	2.98745	8.95087422	100	2938.05575
	Atrazine-desethyl	C6H10N5Cl	187.0624732	6.094857653	15.8475633	76	3985.096867
Point M							
	Cinerin I	C20H28O3	316.203845	3.095867362	15.00008475	86	1476.098575
	Isoprocarb	C11H15NO2	193.1102789	4.164148511	7.890574611	100	15054.38388
	Hexazinone	C12H20N4O2	252.1586261	-0.599479535	9.227317817	89	6932.648071
Point N							
	Isoprocarb	C11H15NO2	193.1102789	4.164148511	7.890574611	100	15054.38388
	Atrazine-desethyl	C6H10N5Cl	187.0624732	0.987554332	14.09568675	98	4309.958675
Point O							
	Cinerin I	C20H28O3	316.203845	3.203775269	14.7736345	90	1380.142701
	Warfarin	C19H16O4	308.1048592	0.645322	13.09685764	98	3456.069687

Graph 2-2: A graph of Isoprocarb detected at various at multiple samples sites for various collection days.

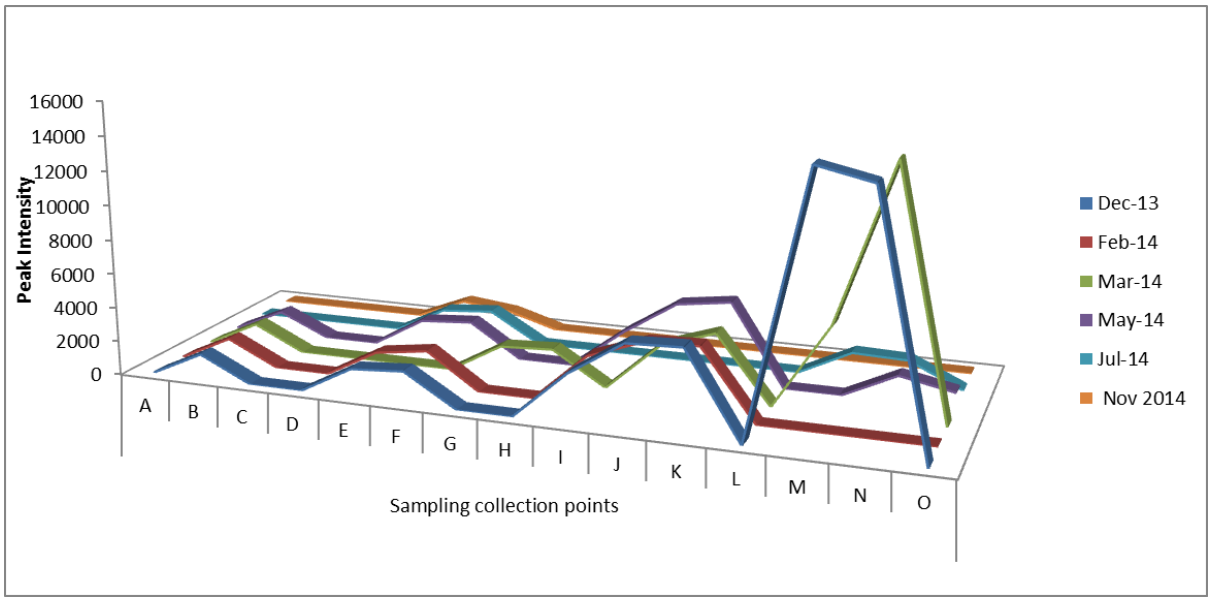


Table 2-8: A summary of all pesticides identified in the Kat River using the Non-targeted approach during the period November 2013-November 2014

	Dec 2013 sample collection														Feb 2014 sample collection														March 2014 Sample collection													
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Atrazine-desethyl			✓					✓						✓	✓			✓						✓				✓																	
Cinerin I							✓			✓			✓									✓				✓													✓			✓			
Cyromazine									✓																✓													✓						
DEET			✓						✓									✓			✓				✓													✓						
Diphenylamine				✓							✓								✓																							✓			
Fenobucarb				✓															✓									✓																
Hexazinone		✓			✓					✓			✓				✓	✓	✓						✓	✓						✓				✓				✓	✓				
Isoprocarb		✓			✓				✓	✓	✓		✓	✓			✓			✓	✓				✓	✓						✓				✓			✓	✓			✓	✓	
Promecarb				✓							✓																✓																
Warfarin			✓				✓								✓							✓																						
3,4,5 Trimethylcarb		✓																																										
Buprimate				✓																																								
Prohexadione				✓																																								
Pyrethrin I							✓																																					
Cinerin II							✓																																					

	May 2014 Sample collection															July 2014 Sample collection													
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Atrazine-desethyl								✓				✓																	✓	
Cinerin I							✓	✓																				✓		✓
Cyromazine																														
DEET						✓			✓											✓										
Diphenylamine				✓							✓																			
Fenobucarb												✓																		
Hexazinone				✓	✓	✓				✓	✓		✓						✓	✓								✓		
Isoprocarb		✓			✓	✓			✓	✓	✓		✓	✓					✓	✓								✓	✓	
Promecarb				✓						✓																				
Warfarin							✓																							✓
3,4,5 Trimethylcarb																														
Buprimate																														
Prohexadione																														
Pyrethrin I																														
Cinerin II							✓																							

The above results were obtained from the analysis of pesticides in Kat River water samples using the non-targeted approach. There were other peaks from the Total Ion Chromatographs which did not match with the pesticides in the targeted screen approach used in section 2.9.1.To identify these peaks, we used the non-targeted approach. Therefore the same TICs (Figure 2-22) used in the targeted approach were also used for the screening of pesticides which might not have been part of the spray regime for the season 2013/2014.The pesticide compounds were detected with the aid of the XIC manager software by running the TICs against the pesticide library (see typical example in Figure 2-23 and more examples in Annex 4).All the pesticides detected were confirmed by applying the same experimental flow diagram as exemplified in Figure 2-24.

The results in Table 2-7 and Table 2-8 (see Annex 4 for results from other sample collection days) show that Isoprocarb was detected at point B, and at successive points E and F ; I, J, K, M and N at increasing concentrations. This means that the sources of Isoprocarb are farms around points B, E, F, I. J, K, M, and N and these farmers were using the same spray regime during the previous seasons such that Isoprocarb is still persistent in the soil hence it is detected in the Kat River water. Alternatively, the source is either the orchards which are not within the Kat River valley on which Isoprocarb pesticide is used for crop pesticide protection hence contaminating Kat River through non-point source pollution or small scale farmers who are using this pesticide for their farming activities.

The results also show that Hexazinone was detected at point B and at successive points E and F but at decreasing concentration. This also applies to Promecarb which was detected at point

D and successive points K and L at decreasing concentrations. The sources of Hexazinone and Promecarb are the farms around points B and E and points D and k respectively. The two pesticides are also persistent and contaminate the river downstream.

The above interpretations on results in Table 2-7 can be explained as follows: either the farmers are using a different spray regime in the 2013/2014 season to the 2012/2013 spray such that some of the pesticides used in the 2012/2013 season degrade slowly hence are detected in the Kat river water or these pesticides are contaminating Kat River through non-point source pollution. The results in Table 2-7 and other results in Annex 4 all support the conclusion that the Kat River is suffering both point and non-point source pollution.

Identification of pesticides contaminants not found in the pesticide commercial library from Kat River water samples.

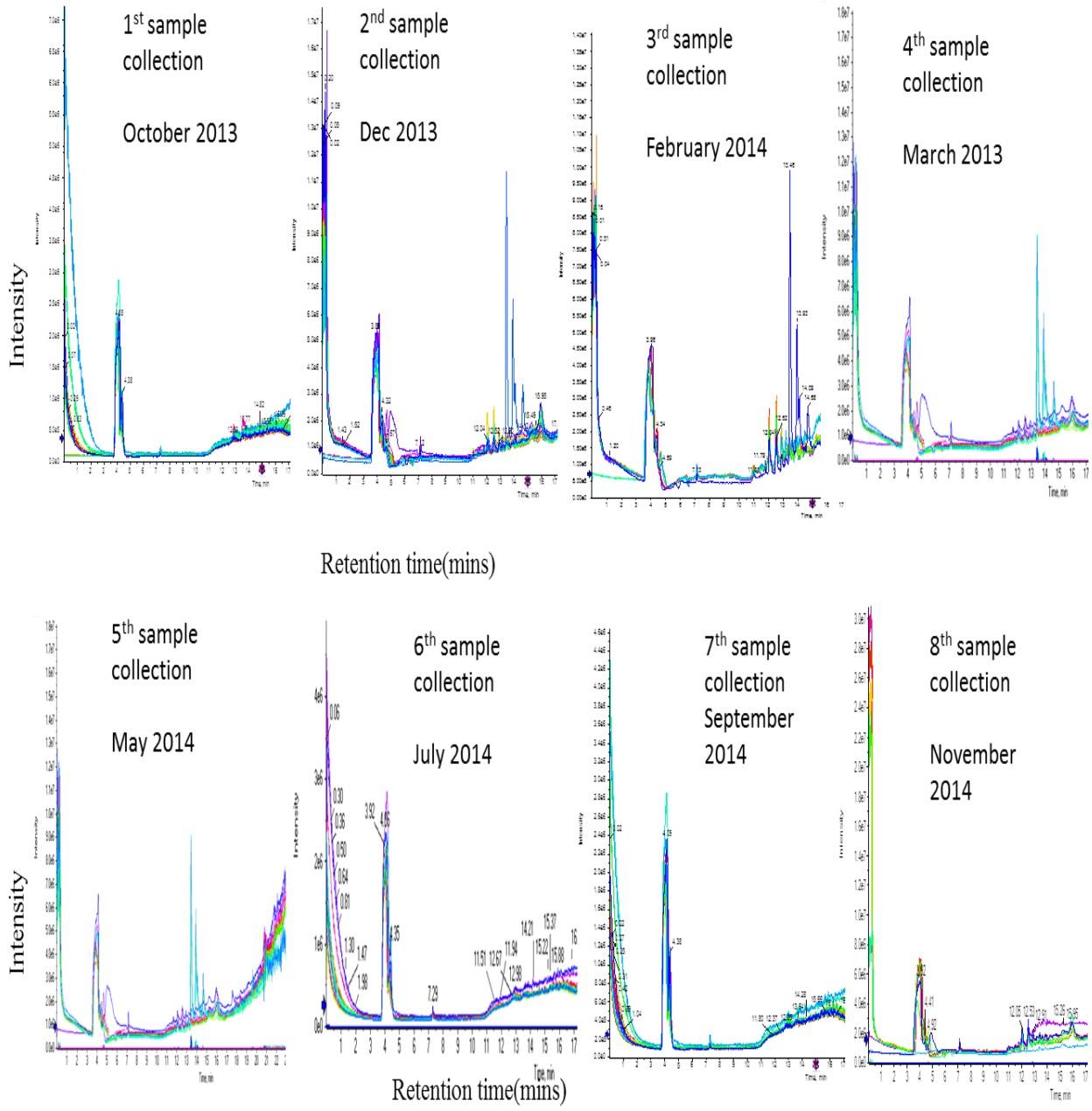


Figure 2-25: Total Ion Chromatography (TIC) obtained by combining all mass spectra from 15 samples collected during the same day, from various points along the Kat River.

Figure 2-26: Typical XIC manager showing the identification of compounds not found in the pesticide library based on the presence of accurate mass molecular ion; characteristic fragment ions in MS/MS mode and isotopic profile.

Figure 2-27: Typical example of pesticides identified using the retrospective approach in Kat River water sample. A compressed experimental flow diagram for Dodemorph (far left); Hexazinone (centre) and Dimethyl carbamat (far right) is displayed.

Table 2-9: Typical example of results obtained from screening pesticide that are not found in the pesticide library.

CompoundName	Formula	ExtractionMass	FoundAtMass	MassError	Retention tim	Intensity
SAMPLE D						
Metolcarb	C9H11NO2	166.0862552	166.0863058	0.304810881	2.898904559	58525.79185
Esprocarb	C15H23NOS	266.1573128	189.1597154	-0.207015709	2.064919463	1175.146746
Isoprocarb	C11H15NO2	194.1175553	194.1169239	-3.25293998	16.46399449	1191.739731
SAMPLE G						
Cymoxanil	C7H10N4O3	199.0825668	199.0799311	-13.23913718	4.180715153	1099.869265
3-Hydroxycarbofuran	C18H35NO	237.123369	237.1230568	-1.316967655	2.904567977	4336.04294
Cartap	C7H15N3O2S2	238.067847	238.0693098	6.144624306	4.192329272	113686.1071
SAMPLE H						
Pirimicarb	C11H18N4O2	239.1502525	239.1491718	-4.519035719	2.965609568	8147.135186
Imazapyr	C13H15N3O3	262.118618	262.1188406	0.849150812	3.426944983	2225.267882
Esprocarb	C15H23NOS	266.1573128	266.1584808	4.388206733	4.538637477	2427.191823
Imazapic	C14H17N3O3	276.1342681	276.1345252	0.931082844	3.476109542	1091.623762
Oxadixyl	C14H18N2O4	279.1339338	279.1339427	0.032111009	2.656960499	8345.962406
3-Hydroxycarbofuran	C18H35NO	282.2791415	282.2797257	2.069722158	14.59071767	35883.54333
SAMPLE I						
Difenoxuron	C16H18N2O3	287.1390191	287.1372338	-6.217739105	4.536695477	1744.480178
Desmedipham	C16H16N2O4	301.1182837	301.1205752	7.609941373	3.829135288	1349.239924
Esprocarb	C15H23NOS	266.1573128	301.1205752	7.609941373	3.829135288	1349.239924
Tetramethrin	C19H25NO4	332.185635	332.1840546	-4.757579177	2.592846258	1094.762252
Benfuracarb	C20H30N2O5S	411.1948207	411.1975129	6.547234565	6.478957746	1760.702064

The results reported in this section were obtained from the analysis of pesticides using the retrospective approach (detection of pesticide compounds which are not in the pesticide library). The same TICs used in section 2.9.1 and 2.9.2 was used to identify the pesticide compound using the retrospective approach (see Figure 2-25).

The pesticide library was not used in this approach. We used the exact accurate masses (Figure 2-26) of specific peaks obtained from the TICs (Figure 2-25) and their fragment ions to identify these pesticide compounds. The ChemSpider website (www.ChemSpider.com) was used for identification of the pesticide compounds (see Figure 2-27 for a summarised experimental flow diagram).

The results obtained (see Table 2-9) showed that Esprocarb detected at point D was also detected downstream at successive points H and I but at lower concentration levels. The sources of Esprocarb are therefore the farms around point D and H. This also means that Esprocarb degrades slowly and hence contaminate the river downstream. However since Esprocab does not form part of the spray regime for the 2013/2014 season, Esprocarb is either a non-point source pollutant contaminating the river through small scale farmers praying their vegetables with unregistered pesticides. The intensity of Isopracarb also showed that high doses of this pesticide is being introduced on the crops and hence into the

environment. Isoprocarb was also consistent with other results obtained from other sample collection days.

3-Hydrocarbofuran used as a typical example in Figure 2-27 was detected at successive points G and H at increasing concentration. This means that the sources of 3-Hydrocarbofuran are farms around points G and H and the farms are using the same spray regime and contaminating the river downstream. Just upstream sampling point H but downstream sampling point G is a tributary pouring into Kat River which we suspect to be heavily contaminated with 3-Hydrocarbofuran hence a huge increase (from 4336.04294 to 35883.54333 see Table 2-9) in the peak intensity of 3-Hydrocarbofuran.

The other pesticides (Table 2-9) detected on other sampling points are not persistent (degrade fast) hence they were not detected downstream and therefore do not contaminate the river downstream. However, some of the pesticides were detected at such high concentration levels that they can cause a huge impact to the environment and communities living around these sampling points. Typical examples of these pesticides detected at high intensities are Primicarb, Metolcarb and Cartap (see Table 2-9).

Fruits and vegetables pesticide analysis

The call to screen for hundreds and thousands of analytes has resulted in the demand for improved extraction methods. Sample preparation and extraction are part of the sophisticated processes involved in the screening of targeted analytes. One of the extraction methods that have gained popularity recently for the extraction of pesticides in food products is the QuEChERS method. This method has the ability to extract pesticides in matrices such as fruits and vegetables and it is based on acetonitrile extraction where the partitioning is achieved by the use of $MgSO_4$. Dispersive solid phase extraction is then applied in the clean-up process. The 5600 AB SCIEX Triple TOF has high sensitivity and selectivity detection. In addition to that, in order to reduce matrix effect during ionisation, sample extracts can be further diluted.

The sample extracts from 5 different fruits and vegetables were run on the 5600 AB SCIEX Triple TOF and the following results were obtained.

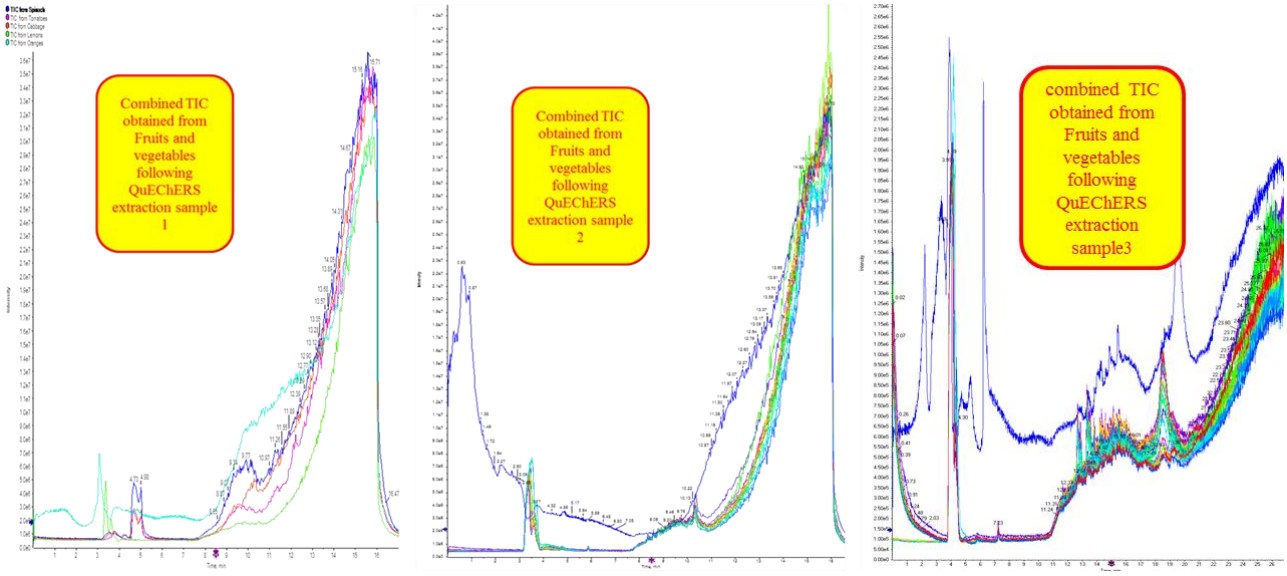


Figure 2-28: TIC obtained from the fruits (left) and vegetables (right) following the QuEChERS extraction method. (1) First sample collection total ion chromatograms representing 5 different fruits and vegetables including lemons, oranges, cabbage, tomatoes. (2) Second sample collection total ion chromatograms of 5 different fruits and vegetables. (3) Third sample collection Total Ion Chromatograms of 5 different fruits and vegetables.

452	☑		Pirimicarb	C11H18N4O:	0	238.14298	+H	239.15025	0.006291782	239.14993	-1.3	0	2	8.59	
53	☑		Buprofezin	C16H23N3O:	0	305.15618	+H	306.16346	0.006291782	306.16271	-2.5	0	2	8.79	
264	☑		Hexazinone	C12H20N4O:	0	252.15863	+H	253.1659	0.006291782	253.16621	1.2	0	2	9.23	
591	☑		DEET	C12H17NO	0	191.13101	+H	192.13829	0.006291782	192.13887	3	0	2	8.5	
94	☑		Diphenylamir	C12H11N	0	169.08915	+H	170.09643	0.006291782	170.0971	4	0	2	15.55	
7	☑		Prohexadione	C10H12O5	0	212.06847	+H	213.07575	0.006291782	213.07475	-4.7	0	2	9.55	
431	☑		Promecarb	C12H17NO2	0	207.12593	+H	208.13321	0.006291782	208.1341	4.3	0	2	8.24	
441	☑		Fenobucarb	C12H17NO2	0	207.12593	+H	208.13321	0.006291782	208.1341	4.3	0	2	8.24	
307	☑		Trinexapac-e	C13H16O5	0	252.09977	+H	253.10705	0.006291782	253.10632	-2.9	0	2	12.32	
145	☑		Benomyl	C14H18N4O:	0	290.13789	+H	291.14517	0.006291782	291.14337	-6.2	0	2	8.96	
296	☑		Dodemorph	C18H35NO	0	281.27187	+H	282.27914	0.006291782	282.28079	5.8	0	2	15.45	

Figure 2-29: Typical example of XIC manager obtained by running the TICs obtains fruits and vegetables against the pesticide library using the target screen approach

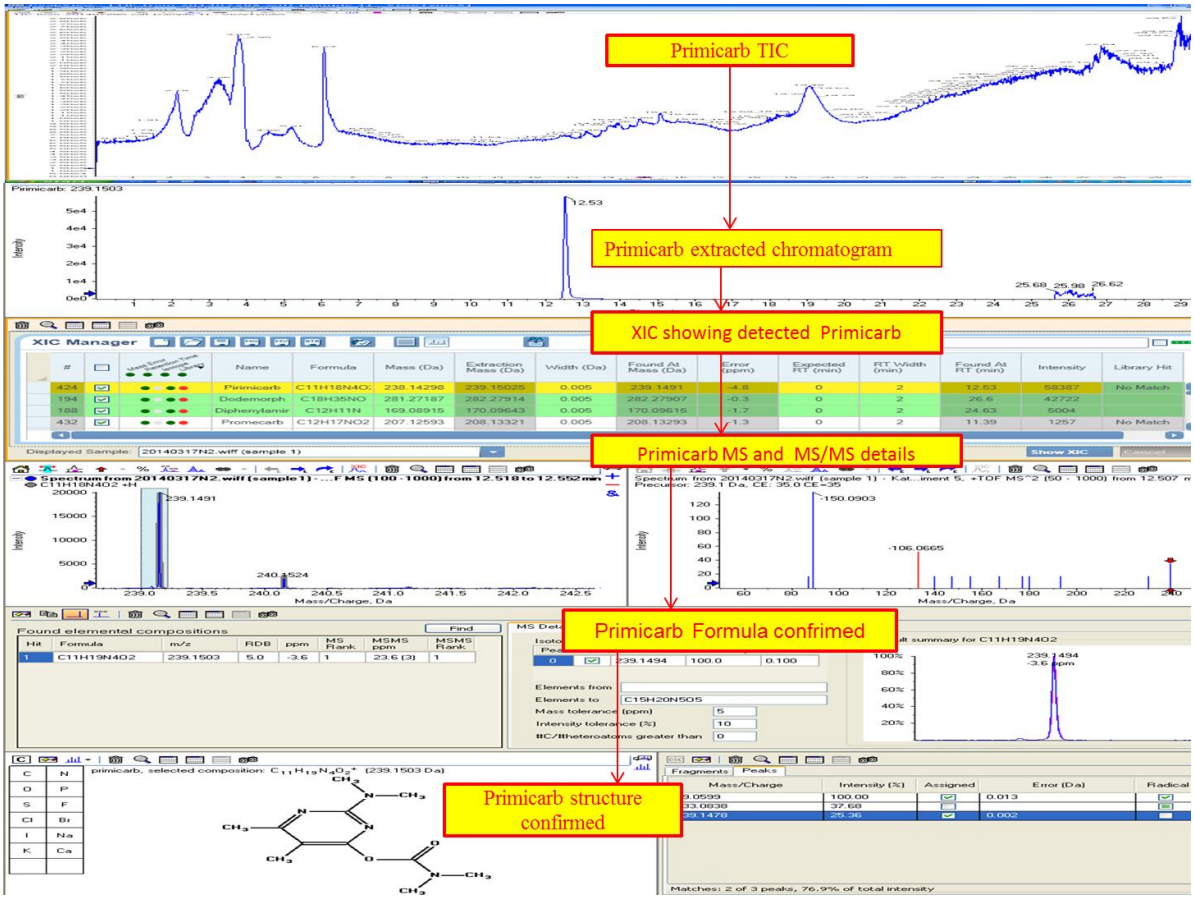
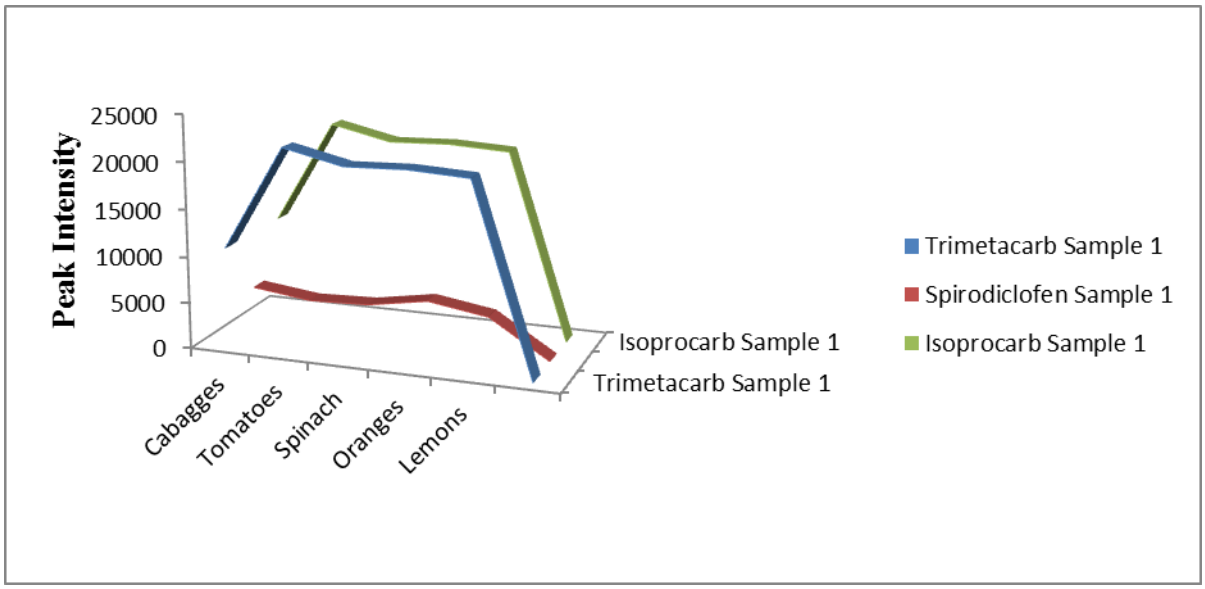


Figure 2-30: Typical example of experimental flow diagram for the identification and confirmation of Primicarb in an Orange sample after running the TIC obtained from an Orange sample against the pesticide library.

Table 2-10: Fruits and vegetable analysis - Target compounds identified at different F&V samples Tomatoes; Lemons; Oranges; Spinach and Cabbage) collected from both commercial and small scale farmers.

Tomatoes						
Diphenylamine	C12H11N	169.0891494	-2.71202542	15.46822425	0.866981216	48602.61032
Simazine-2-hydroxy	C7H13N5O	183.1120102	15.86505516	8.955486314	0.211514386	1567.346058
Isoprocarb	C11H15NO2	193.1102789	-1.714948776	7.975738642	0.452569768	15059.25062
Pirimicarb	C11H18N4O2	238.142976	0.905863429	3.089705177	0.417516933	2183.015959
Cabbage						
Fenazaquin	C20H22N2O	306.1732135	1.470837786	9.76802293	0.843829354	2310.514846
Diphenylamine	C12H11N	169.0891494	-1.408138817	15.85435633	0.871111141	3348.423569
Metolcarb	C9H11NO2	165.0789787	1.089880658	4.539471858	0.303960485	3068.841777
Isoprocarb	C11H15NO2	193.1102789	-0.424296923	7.935334476	0.451229726	11597.94102
Mepronil	C17H19NO2	269.141579	0.613409392	9.675340376	0.268410282	3212.581671
Maleic hydrazide	C4H4N2O2	112.0272775	-1.657333187	4.576250444	0.322139861	16715.71348
Pirimicarb	C11H18N4O2	238.142976	-2.85103234	8.713755062	0.390677631	1103.71153
Pyracarbolid	C13H15NO2	217.1102789	1.986218713	8.094040434	0.249493119	1559.943324
DEET	C12H17NO	191.1310143	-0.395820532	5.024758915	0.336827559	8071.357499
Lemons						
Diphenylamine	C12H11N	169.0891494	-0.225353703	15.87623447	0.925815557	2141.072069
Prosulfocarb	C14H21NOS	251.1343863	8.500164636	3.903924608	0.512983905	2279.138349
Isoprocarb	C11H15NO2	193.1102789	-0.712745932	7.957379613	0.452931391	14245.00599
Pirimicarb	C11H18N4O2	238.142976	-5.638512787	8.682316715	0.271629864	7737.225177
DEET	C12H17NO	191.1310143	-1.346912681	8.572322881	0.258029164	5725.50297
Oranges						
Buprofezin	C16H23N3OS	305.1561844	-5.453329663	8.890305566	0.447476776	2641.678763
Dodemorph	C18H35NO	281.271865	3.258990466	15.21449374	0.554335756	94546.27951
Isoprocarb	C11H15NO2	193.1102789	2.551409631	7.953782817	0.45837005	13386.97468
Promecarb	C12H17NO2	207.125929	3.553771941	5.021376051	0.108909319	2263.910242
Fenobucarb	C12H17NO2	207.125929	3.553771941	5.021376051	0.108909319	2263.910242
Pirimicarb	C11H18N4O2	238.142976	-1.53962314	4.798579569	0.520942215	1385.721989
Mexacarbate	C12H18N2O2	222.136828	-9.161692038	9.864269498	0.242743276	3201.248541
DEET	C12H17NO	191.1310143	1.021308615	5.029341296	0.339817543	5089.524727
Triphenylphosphate	C18H15O4P	326.0707976	2.692349236	13.58275809	0.959103971	1706.545493
Spinanch						
Cinerin II	C21H28O5	360.1936743	-3.06284793	13.19328057	0.91818575	7043.067133
Fenazaquin	C20H22N2O	306.1732135	3.212735294	9.754228051	0.76698791	6290.810646
Dodemorph	C18H35NO	281.271865	3.13890225	14.91781592	0.388246215	85627.38292
Fluazifop (free acid)	C15H12F3NO4	327.0718428	5.0435152	10.27397004	0.693437295	285076.5769
Isoprocarb	C11H15NO2	193.1102789	1.458159982	7.929014014	0.449592064	10736.79269

Graph 2-3 : A graph showing three pesticides detected in different fruits and vegetables samples from first sample collection.



The total ion chromatograms in Figure 2-28 were obtained from five different fruits and vegetables collected from farmers within the research area. The fruits were from commercial farms supplying their products at local and international level, while the vegetables were obtained from small scale farmers. The TIC of an orange sample from third sample collection was run against the pesticide library. All the three approaches were used to identify and confirm the presence of pesticides in 5 different fruits and vegetables. See Figure 2-30 for typical experimental flow diagram. Typical example of combined results obtained from a single sample collection analysed using the three approaches is displayed in the XIC manager (see Figure 2-29).

Firstly, the results obtained (see Table 2-10 for typical example of results and Annex 5 for more results), confirmed the presence of pesticides in fruits and vegetables detected in the Kat River. The table of results show the identification of the following pesticides both in Kat River water and fruits (Oranges and lemons) by the targeted screen approach: Spirodiclofen, Pryriproxyfen, imidacloprid, imidacloprid and Primicarb whereas Hexazinone and Isoprocab are the confirmed pesticides which were detected both in Kat River and fruits(Oranges and Lemons) by the non-targeted screen approach. The source of pesticide detected in the Oranges and Lemons using the targeted approach are the farms which are using these pesticides in their spray regime. This also means that these pesticides were persistent on the fruits (Oranges and lemons) and were detected as residues. Pesticides identified in Oranges and Lemons using the targeted approach were detected at a higher intensity compared to the

same pesticides detected in Kat River by the same screening approach. The main reason for the difference in the intensities between the pesticides detected in Kat River water samples compared to those detected in fruits (See Table 2-5 and Table 2-10 for pesticide intensity comparison; and Annex 4 and Annex 5 for other results comparison), is that pesticides in the river water are more diluted compared to those in the fruits. The same applies to vegetables samples used in this research.

The three approaches used for the detection of pesticides confirmed the presence of pesticides both in Kat River water samples and vegetable samples. For instance, Pryproxifen was detected in both Kat River water samples and Vegetables (Spinach and Cabbages) using the targeted screen approach in both scenarios. In this case, the sources of Pyriproxyfen contaminating the vegetables are the farms upstream the vegetable gardens that were using Pyriproxyfen in their spray regime. The same applies to Spirodiclofen detected both in Kat river water samples and vegetables (Spinach Cabbages) (see Table 2-5 and Table 2-7)

On the other hand, pesticides such as Dodemoph (Spinach and Cabbage), 3-Hydrocarbofuran (Tomatoes) and Isoprocarb (Tomatoes and Cabbage) were also detected in Kat River water samples using the non-targeted approach and the identification of compounds not in the library approach.However, none of the compounds were part of the spray programme. Therefore, the source of these contaminants is the small scale farmers spraying high doses of these pesticides on their vegetables. This also means that these pesticides degrade slowly and contaminate both the vegetables and river downstream.

There is an exception of pesticides detected in the vegetables and never detected in the Kat River samples. Typical examples of these pesticides are Metolcarb detected in Tomatoes and Fenazaquin detected in Spinach. The source of Metolcarb and Fenazaquin is the small scale vegetable gardens owners. However, Metolcarb and Fenazaquin are not persistent and do not contaminate the river downstream hence were not detected in the Kat River water.

There were no pesticides detected in vegetables and Kat River water samples which were also detected of fruits (Oranges and Lemons).

3 CHAPTER THREE

PHARMACEUTICAL AND PERSONAL CARE PRODUCTS (PPCPS) ANALYSIS

Introduction

There is growing concern that commonly used Pharmaceuticals and Personal Care Products (PPCP) are entering and contaminating drinking water supplies. The use of targeted quantitation of PPCP has been well established but there is an emerging trend to also screen for and identify unexpected environmental pollutants. Chemicals like hormones and antibiotics are especially of interest because of proven endocrine disrupting effects and a possible development of bacterial resistance. Powerful screening methods are required to detect and quantify the presence of these compounds in our environment. PPCP encompass a wide range of pollutants, including Endocrine Disrupting Compounds (EDC), pesticides, hormones, antibiotics, drugs of abuse, x-ray contrast agents and drinking water disinfection by-products to name a few. In order to properly assess the effects of these compounds on our environment, it is necessary to accurately monitor their presence. The diversity of chemical properties of these compounds makes method development challenging. LC/MS/MS is able to analyse polar, semi-volatile, and thermally labile compounds covering a wide molecular weight range.

The new AB SCIEX TripleTOF™5600 LC/MS/MS is used to profile environmental samples for unexpected pollutants, to identify and characterise the chemical composition and structure of the pollutants, and to confirm their presence in collected water samples. Liquid Chromatography coupled to tandem Mass Spectrometry (LCMS/ MS) is able to analyse polar, semi-volatile, and thermally labile compounds covering a wide molecular weight range, such as pesticides, antibiotics, drugs of abuse, x-ray contrast agents, drinking water disinfection by-products etc. More recently there is a growing interest from environmental researchers to also screen for and identifies non-targeted compounds in environmental samples, including metabolites and degrades, but also completely unexpected pollutants. The new AB SCIEX TripleTOF™5600 LC/MS/MS system is capable of performing highly sensitive and fast MS scanning experiments to search for unknown molecular ions while also

performing selective and characteristic MS/MS scanning for further compound identification and, therefore, is the instrument of choice for this challenging task.

3.1 Materials and methods

Chemicals

Methanol, water and acetonitrile reagents were purchased from Separations (SA), while the formic acid (analytical grade) was purchased from Agilent, USA. The Quenchers was purchased from Separations (SA). The LC–MS column was purchased from Agilent (USA). APCI Negative calibration solution and APCI Positive calibration solutions were purchased from Separations (SA). All reagents and standard were prepared and ready for analytical use.

3.2 Sampling and sample preparation

Water samples were collected using 500ml amber glass bottles Samples were collected upstream and downstream the two WWTPs (Seymour and Fort Beaufort) and the behaviour of PPCPs monitored and compared. Also samples were collected between the two WWTPs (before and after every community) as they are 46km apart and both discharge in Kat River. Samples were collected from points along Kat River from as close to the source as possible going downstream Samples were collected from November 2013 to November 2014. The sampling points and their coordinates are shown in Table 3-1.

Table 3-1: PPCPs sampling points and their coordinates

Sampling point and description	Coordinates
Point A(Control and Upstream Seymour WWTP)	(-32.538729;26.765033)
Point B(downstream Seymour WWTP)	(-32.548352;26.764319)
Point C(Upstream Kat River Dam)	(-32.552227;26.766631)
Point D (Kat River Dam)	(-32.561004;26.771847)
Point E(Before Balfour community)	(-32.555613;26.690156)
Point F(After Balfour community)	(-32.563859;26.77314)
Point G(Before Fort Beaufort town)	(-32.772922;26.630673)
Point H(After Fort Beaufort town)	(-32.796837;26.617634)

Point I(Upstream Fort Beaufort WWTP)	(-32.777643;26.645549)
Point J(Downstream Fort Beaufort WWTP)	(-32.786208;26.645549)

The direct injection method was used for introducing the samples for analysis with the liquid chromatograph couple to tandem mass spectrometry. The water samples were first centrifuged at 13400Xg before filtered through filtration cellulose acetate membrane filters (0.45um) and loaded directly into 1.5ml sample vials. All water samples were brought to the laboratory in ice-cubes and were analysed immediately.

3.3 Apparatus and Chromatographic conditions

The chromatographic conditions and apparatus were set up using the same conditions as described in section 2.6 of Chapter 2.

3.4 Data analysis

Data analysis was performed using the same protocols as described in section 2.7.2 using the non-targeted approach.

3.5 Results and Discussion

The modern tool that can be used for the screening of PPCPs is none other than LC/MS. This is because of its ability to combine the high resolution property of the LC separation to the high sensitivity MS/MS when being operated in MRM mode. In this study we applied the LC/MS technique to screen for pharmaceuticals and personal care products. PPCPs are of growing concern because they are biologically active compounds with unknown effects in the long run, bio accumulate and resist degradation in WWTPs. With two WWTPs within our research area which have been recently reported not to be fully functional, there was need to screen for PPCPs and their potential effects to the general public.

Total Ion Chromatograms (TICs) were created by summing up intensities of all mass spectral peaks belonging to the same scan. Each scan consisted of 10 samples collected from Kat River at key samplings points. The Direct injection method was used at 200µl.The control (blank) is also shown.

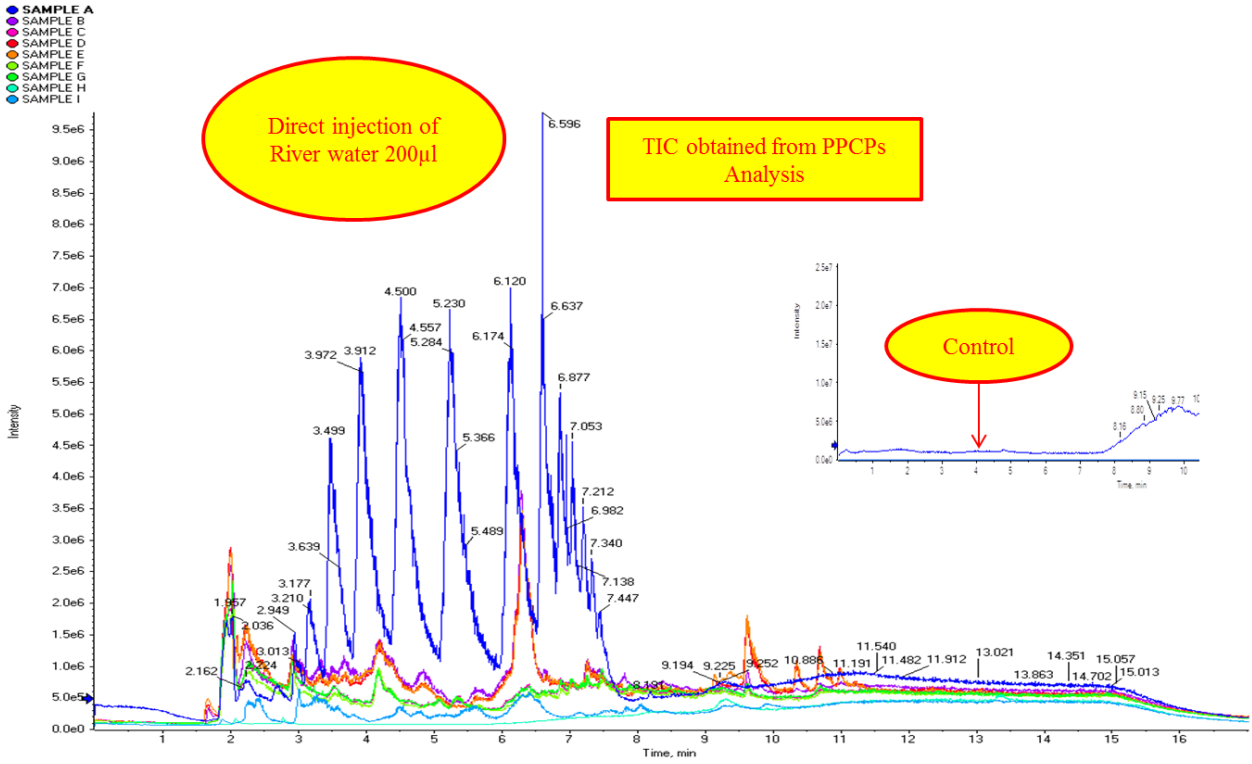


Figure 3-1: Total Ion Chromatography (TIC) obtained by combining all mass spectra from 8 samples collected during the same day, from various points along the Kat River.

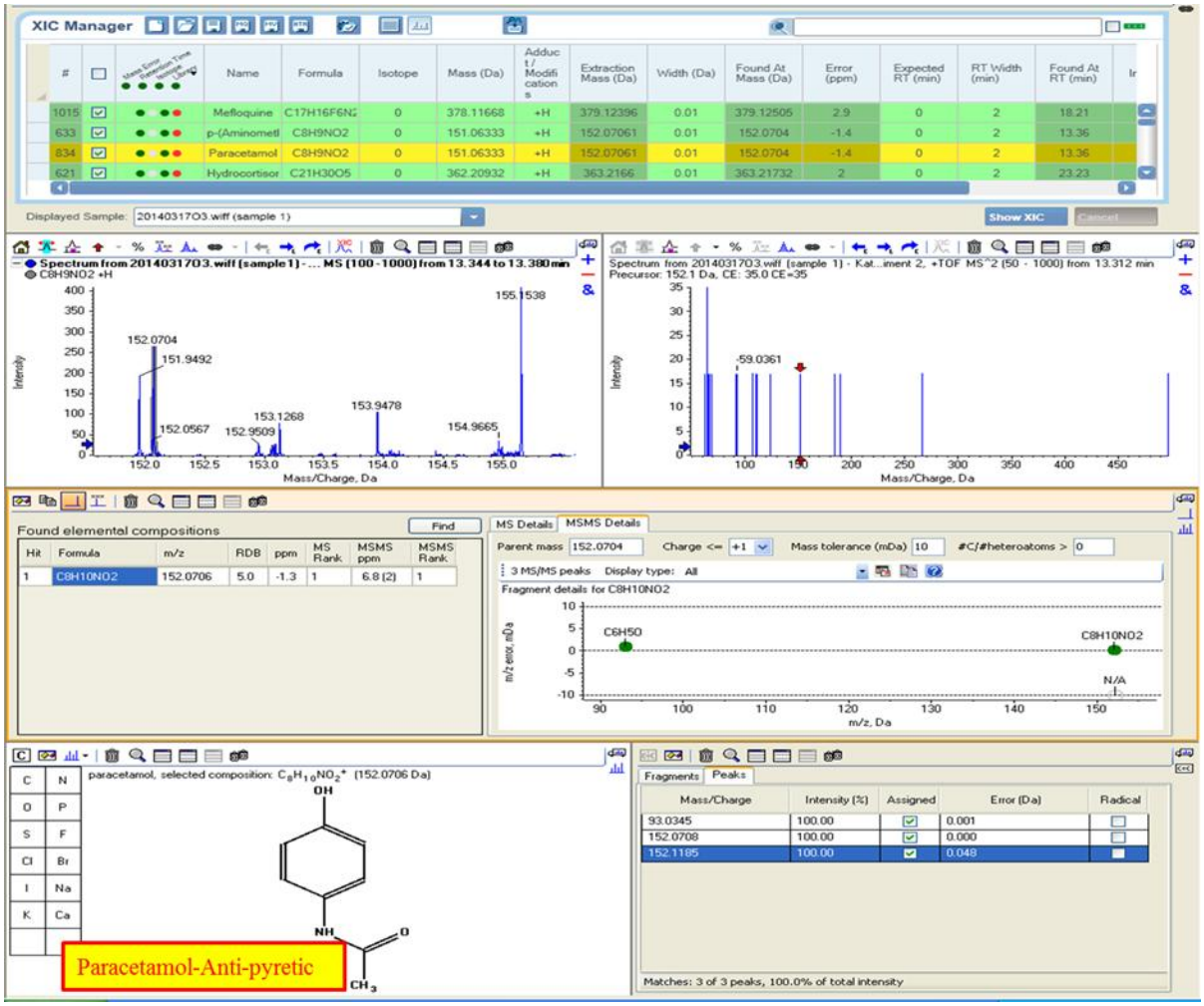


Figure 3-2: Typical example of compressed experimental flow diagram for Paracetamol identified using the non-targeted screen approach.

Table 3-2: Typical example of Pharmaceuticals and Personal care Products identified using the non-targeted screen approach.

Index	CompoundName	Formula	Mass	Mass error	Retention time	LibraryScore	Intensity
Sample B							
	Dixyrazine	C24H33N3O2S	427.2293494	-7.822556457	9.667294087	0.455100275	4053.153813
	Timolol	C13H24N4O3S	316.1569127	-5.769483037	13.92992174	0.608710863	2803.170907
	Ajmaline	C20H26N2O2	326.1994283	-10.42771629	8.932425775	0.738314554	10144.55741
	Nomifensine	C16H18N2	238.1469987	-16.51742631	8.596131133	0.217347432	26693.24881
	Diaveridine	C13H16N4O2	260.127326	-5.416989933	8.607999555	1	293241.4512
	Gabapentin	C9H17NO2	171.125929	4.728845915	10.38843874	0.140005132	1341.014116
	Biotin	C10H16N2O3S	244.0881644	245.0998074	17.81575188	9.122281143	52672.89054
	Coniine	C8H17N	127.1360997	4.223670924	8.585081221	0.420443935	3077.071431
	Buprofezin	C16H23N3OS	305.1561844	-2.376983826	8.786965649	0.447476776	35106.75965
	Paracetamol	C8H9NO2	151.0633286	0.0100045	13.9874494	0.8887645	4045.875644
	Carbimazole	C7H10N2O2S	186.0462995	24.06888484	8.034755402	0.170333275	4490.725141
	3,4-Methylenedioxymethamphetamine	C11H15NO2	193.1102789	1.913361173	7.883729155	0.456168381	16220.08125
	Primidone	C12H14N2O2	218.1055279	-7.590997888	8.368627492	0.20956766	4893.024409
	N,N-Diethyl-m-toluamide	C12H17NO	191.1310143	4.26628933	8.474701563	0.240790702	7663.363712
	Hydroxymethylpyridine	C6H7NO	109.0527639	3.848369273	5.840794696	0.773264413	1013.482763
	Metamfepramone	C11H15NO	177.1153642	2.734446148	8.206320913	0.162300608	8179.829194
	Sulfaquinoxaline	C14H12N4O2S	300.0680976	5.39553541	13.69293976	0.928754734	2447.994456
	Cyamemazine	C19H21N3S	323.1456197	-1.440129214	15.12853796	0.808019442	85691.60239
	Pindolol	C14H20N2O2	248.1524781	-5.586016819	9.212584955	0.604766698	5691.182578
	Metamitron	C10H10N4O	202.0854611	-13.85631001	7.471386308	0.797294894	2672.320391
	Oxitropium	C19H26NO4	332.1861836	-9.737333638	9.72712898	0.828013195	8217.306602
Sample D							
	Dixyrazine	C24H33N3O2S	427.2293494	-7.214731196	9.674836898	0.522174937	3511.972777
	Irbesartan	C25H28N6O	428.2324598	-0.687919801	16.04240045	0.683854593	2880.307565
	Timolol	C13H24N4O3S	316.1569127	-3.348135085	13.90910948	0.552692832	5625.099091
	Ajmaline	C20H26N2O2	326.1994283	-10.01278593	8.939799639	0.584290633	8208.276985
	Nomifensine	C16H18N2	238.1469987	-17.63574169	8.608561252	0.206492025	33008.48382
	Diaveridine	C13H16N4O2	260.127326	-6.303447901	8.629427032	1	336712.0839
	Phencyclidine	C17H25N	243.1987	4.472685369	12.19349442	0.474000034	1247.52969
	Mafenide	C7H10N2O2S	186.0462995	25.43315919	8.032960396	0.170333275	5912.705058
	Coniine	C8H17N	127.1360997	2.721406888	8.579076866	0.430321964	2705.283178
	Betaine	C5H11NO2	117.0789787	2.290732109	3.939548322	0.846575673	1598.042239
	Dihydralazine	C8H10N6	190.0966944	15.17530969	15.09546709	0.130177928	6360.422298
	Perazine	C20H25N3S	339.1769198	-8.95212118	9.50746512	0.237677299	2574.476084
	Tocainide	C11H16N2O	192.1262633	-7.622345509	11.97264797	0.714900965	1835.784055
	Paracetamol	C8H9NO2	151.0633286	0.0100045	13.9874494	0.8887645	4045.875644
	Bunitrolol	C14H20N2O2	248.1524781	-3.506577709	9.208119368	0.604766698	4542.999102
	Oxprenolol	C15H23NO3	265.1677938	-4.286959478	12.16985552	0.679496797	1236.292181
	Carbimazole	C7H10N2O2S	186.0462995	25.43315919	8.032960396	0.170333275	5912.705058
	3,4-Methylenedioxymethamphetamine	C11H15NO2	193.1102789	4.191347237	7.890232458	0.455789071	17785.50013
	Primidone	C12H14N2O2	218.1055279	-9.568949062	8.380806586	0.20956766	6141.887037
	Trimethoprim	C14H18N4O3	290.1378907	-5.267151901	8.956132272	0.494224432	5553.710686
	Biotin	C10H16N2O3S	244.0881644	245.0998074	17.81575188	9.122281143	52672.89054
	Metamfepramone	C11H15NO	177.1153642	3.777819394	8.21249553	0.152304522	8478.806627
	Tetroxoprim	C16H22N4O4	334.1641055	-5.269123898	8.974579919	0.854426811	2019.797998
	Hexazinone	C12H20N4O2	252.1586261	-0.599479535	9.228548294	0.121990529	8115.122083
	Sulfaquinoxaline	C14H12N4O2S	300.0680976	2.711692731	13.67508584	0.928754734	2540.732602
	9-Hydroxyrisperidone	C23H27FN4O3	426.2067193	-6.025010479	14.84094332	0.872181828	6269.433633
	Pindolol	C14H20N2O2	248.1524781	-3.506577709	9.208119368	0.604766698	4542.999102
	Metamitron	C10H10N4O	202.0854611	-14.38245657	7.487154868	0.749338898	4553.592005
	Oxitropium	C19H26NO4	332.1861836	-8.828646796	9.818176517	0.828013195	7869.787297

Sample F							
	Timolol	C13H24N4O3S	316.1569127	-9.718339931	13.92355448	0.437487856	3578.953891
	Cefepime	C19H24N6O5S2	480.1249618	0.765954536	12.54753705	0.146702628	1265.187105
	Nomifensine	C16H18N2	238.1469987	-19.8701252	8.602870961	0.248935619	32278.56502
	Diaveridine	C13H16N4O2	260.127326	-9.599825589	8.618187341	1	331579.376
	Phencyclidine	C17H25N	243.1987	3.461776067	12.22858943	0.469196356	1267.288564
	Mafenide	C7H10N2O2S	186.0462995	21.51725241	8.040256604	0.158408018	5894.73519
	Coniine	C8H17N	127.1360997	-0.920118657	8.558330119	0.439694907	2608.569288
	Bunitrolol	C14H20N2O2	248.1524781	-6.451431785	9.199196295	0.604766698	4313.748868
	Carbimazole	C7H10N2O2S	186.0462995	21.51725241	8.040256604	0.158408018	5894.73519
	3,4-Methylenedioxymethamphetamine	C11H15NO2	193.1102789	0.410640192	7.900005807	0.449936903	16728.75855
	Primidone	C12H14N2O2	218.1055279	-12.62288518	8.368122592	0.20956766	5937.767456
	Trimethoprim	C14H18N4O3	290.1378907	-8.546534611	8.945145746	0.463913542	2355.129381
	Biotin	C10H16N2O3S	244.0881644	245.0998074	17.81575188	9.122281143	52672.89054
	Metamfepramone	C11H15NO	177.1153642	0.353775913	8.208002127	0.176389609	8892.603688
	Tetroxoprim	C16H22N4O4	334.1641055	-7.553477174	8.88276021	0.854426811	1993.046165
	Desipramine	C18H22N2	266.1782989	-18.18440608	9.712944096	0.108303227	4296.067145
	Cyclicine	C18H22N2	266.1782989	-18.18440608	9.712944096	0.108303227	4296.067145
	Dimefuron	C15H19ClN4O3	338.1145684	1.537318875	15.12005365	0.559062763	9255.952425
	Sulfaquinoxaline	C14H12N4O2S	300.0680976	0.703390865	13.66868357	0.891774015	3123.298118
	9-Hydroxyrisperidone	C23H27FN4O3	426.2067193	-7.376858819	14.83134603	0.8544731	11608.35026
	Pindolol	C14H20N2O2	248.1524781	-6.451431785	9.199196295	0.604766698	4313.748868
	Metamitron	C10H10N4O	202.0854611	-17.47982203	7.459598283	0.80678324	4389.468234
	Oxitropium	C19H26NO4	332.1861836	-11.90822947	9.817310894	0.828013195	11055.82787
	Paracetamol	C8H9NO2	151.0633286	0.0100045	13.9874494	0.8887645	4045.875644
Sample G							
	Tocainide	C11H16N2O	192.1262633	11.9836084	0.506952304	0.325906214	1308.569251
	Fludrocortisone Acetate	C23H31FO6	422.2104673	9.12581511	1	0.110054512	1323.837128
Sample H							
	Timolol	C13H24N4O3S	316.1569127	-11.18092407	13.9133195	0.677718503	3010.372187
	Ajmaline	C20H26N2O2	326.1994283	-12.19659842	8.932212565	0.750374215	8468.213814
	Cefepime	C19H24N6O5S2	480.1249618	0.586090459	12.51626179	0.146702628	1728.494079
	Nomifensine	C16H18N2	238.1469987	-20.45557165	8.591265511	0.218148579	33645.87407
	Diaveridine	C13H16N4O2	260.127326	-9.647004112	8.595451226	1	364393.7974
	Phencyclidine	C17H25N	243.1987	1.060626793	12.23331527	0.448396762	1107.900574
	Mafenide	C7H10N2O2S	186.0462995	22.22227542	8.034857569	0.170333275	4755.337737
	Coniine	C8H17N	127.1360997	-0.423845081	8.570699522	0.325906214	2825.270519
	Terbutryn	C10H19N5S	241.1361176	12.99130832	15.10823301	0.110054512	7943.054323
	Paracetamol	C8H9NO2	151.0633286	0.0100045	13.9874494	0.8887645	10789.09865
	Perazine	C20H25N3S	339.1769198	-11.3723962	9.511252723	0.116200949	2533.910281
	Tocainide	C11H16N2O	192.1262633	-12.75351911	11.96695027	0.525126923	1745.01441
	Bunitrolol	C14H20N2O2	248.1524781	-6.782245699	9.208453391	0.604766698	5475.005482
	Carbimazole	C7H10N2O2S	186.0462995	22.22227542	8.034857569	0.170333275	4755.337737
	Lisinopril	C21H31N3O5	405.2263715	-0.733677831	14.80960167	0.803529263	1964.963378
	3,4-Methylenedioxymethamphetamine	C11H15NO2	193.1102789	0.389312221	7.884310771	0.474284398	16418.71349
	Trimethoprim	C14H18N4O3	290.1378907	-9.241490926	8.964096366	0.686525688	2814.188882
	Biotin	C10H16N2O3S	244.0881644	245.0998074	17.81575188	9.122281143	60786.98576
	Hydroxymethylpyridine	C6H7NO	109.0527639	0.930225708	5.847674169	0.848874513	1864.147854
	Metamfepramone	C11H15NO	177.1153642	0.139905923	8.217013232	0.167937053	8326.099545
	Tetroxoprim	C16H22N4O4	334.1641055	-8.095251124	8.857756771	0.854426811	1835.201202
	9-Hydroxyrisperidone	C23H27FN4O3	426.2067193	-8.942056852	14.83852618	0.828103793	11415.99678
	Pindolol	C14H20N2O2	248.1524781	-6.782245699	9.208453391	0.604766698	5475.005482
	Hydrocortisone 21-acetate	C23H32O6	404.2198891	1.101641921	14.89072267	0.661171046	7961.881056
	Metamitron	C10H10N4O	202.0854611	-17.53532629	7.502274066	0.861377334	3547.844481
	Oxitropium	C19H26NO4	332.1861836	-11.96120345	9.823326821	0.828013195	11443.99904
	Dixyrazine	C24H33N3O2S	427.2293494	-7.607479963	9.668717992	0.313061838	3376.307286
	Fluconazole	C13H12F2N6O	306.1040657	15.94649863	11.75644773	0.31409979	3796.428046

Graph 3-1: Graph showing the intensity of Paracetamol at various sample collection points from three different sample collections

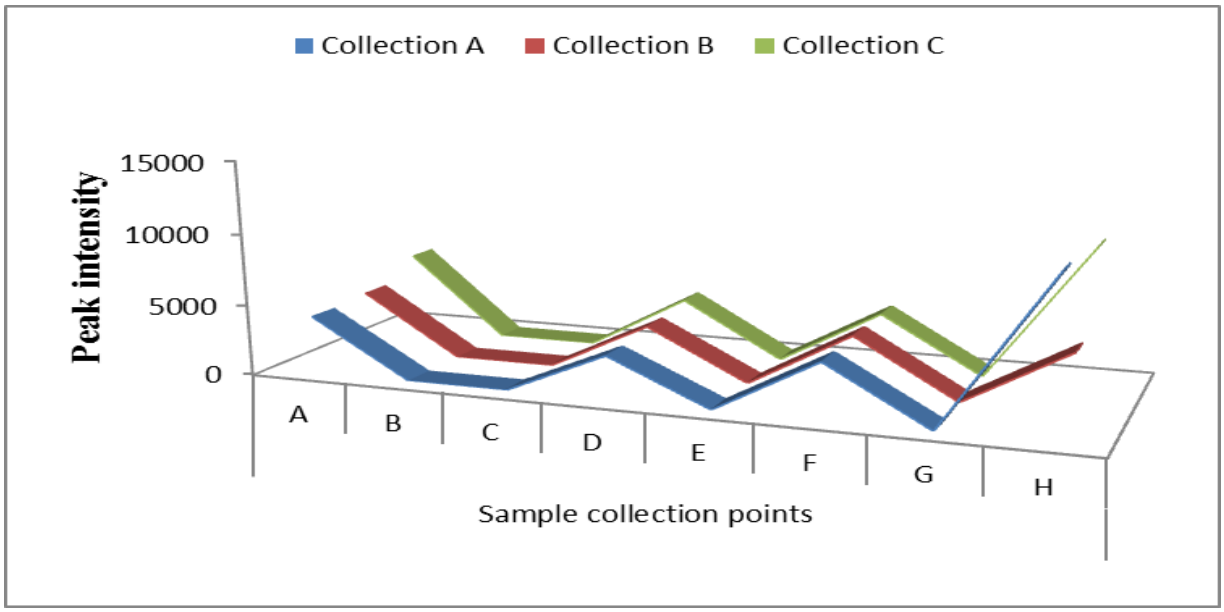


Table 3-3: A summary of all the Pharmaceuticals and Personal Care Products identified in Kat River for the period November 2013- November 2014

	First sample collection								Second sample collection								Third sample collection							
	A	B	C	D	E	F	G	H	A	B	C	D	E	F	G	H	A	B	C	D	E	F	G	H
3,4-Methylenedioxymethamphetamine		✓		✓		✓		✓							✓									✓
9-Hydroxyrisperidone				✓		✓		✓								✓								
Ajmaline		✓		✓				✓																
Betaine				✓										✓										
Biotin		✓				✓		✓		✓								✓		✓			✓	✓
Bunitrolol				✓		✓		✓																
Buprofezin		✓						✓																✓
Carbimazole		✓		✓		✓		✓						✓										✓
Cefepime						✓																		
Coniine		✓		✓		✓		✓		✓														✓
Cyamemazine		✓																					✓	
Cyclicine						✓																		
Diaveridine		✓	✓	✓		✓		✓												✓				
Dihydralazine				✓										✓										
Dimefuron						✓																		
Dixyrazine		✓		✓				✓		✓								✓						
Fluconazole								✓																
Fludrocortisone Acetate							✓																	
Gabapentin		✓																		✓				
Hydrocortisone 21-acetate								✓																
Hydroxymethylpyridine		✓						✓																
Irbesartan				✓						✓														
Lisinopril																								
Mafenide				✓		✓		✓												✓				
Metamfepramone		✓				✓		✓				✓								✓				
Metamitron		✓		✓		✓		✓								✓							✓	
N,N-Diethyl-m-toluamide		✓																						
Nomifensine		✓		✓		✓		✓				✓								✓				
Oxitropium		✓		✓				✓															✓	
Oxprenolol				✓		✓								✓		✓								
Paracetamol		✓		✓		✓		✓		✓		✓		✓		✓		✓					✓	✓
Perazine				✓				✓						✓										
Phencyclidine				✓		✓		✓																
Pindolol		✓		✓		✓		✓		✓		✓		✓		✓		✓					✓	
Primidone		✓		✓		✓									✓									
Sulfaquinoxaline		✓		✓		✓										✓							✓	
Terbutryn								✓																
Tetroxoprim						✓		✓							✓									
Timolol		✓				✓		✓		✓								✓						
Tocainide				✓			✓	✓						✓										
Trimethoprim				✓		✓		✓				✓												
Desipramine						✓																		

After performing a library search of the TIC (see Figure 3-1 and Annex 6 for more results) obtained from the PPCPs scan, an interesting trend was observed from the selected key sampling points. The Key sampling points selected were sampling point B (Downstream Seymour WWTP), sampling point D (Kat River Dam); sampling point E (Before Balfour

community); sampling point H (After Fort Beaufort town) and sampling point J (downstream Fort Beaufort town).The finding are shown in Table 3-2 (see Annex 6 for me results).

To confirm the presence of potential Pharmaceutical and Personal Care Products (PPCPs), the TICs were run against the library and a typical example for the experimental flow diagram for the identification of Paracetamol is displayed in Figure 3-2.

The results obtained (see Table 3-2, Table 3-3, Graph 3-1 and Annex 6 for more results) from the analysis of PPCPs showed that there is high contamination of PPCPs in the Kat River water. Paracetamol, a common over the counter anti-pyretic drug, was detected at high intensities at sample point B (downstream Seymour waste water treatment plant) but at decreasing concentration up until point E (just before Balfour community. The concentration increased again at point F (just downstream Balfour community) and was detected at decreasing concentration between Balfour and Fort Beaufort town. The highest peak intensities were detected at increasing concentrations from at successive points H and J (after Fort Beaufort town and Fort Beaufort WWTP respectively).The increase in intensities of Paracetamol at these key sampling points shows that the Seymour waste water treatment plant, Balfour community and Fort Beaufort town and waste water treatment plant are the main sources of these PPCPS. The PPCPs are persistent (degrade slowly) and are contaminating the river downstream. The same applies to Biotin, Timolol (commonly used for treating glaucoma, heart attacks, hypertension, and migraine headache) and Coniine (a neurotoxin which disrupts the peripheral nervous system).The sources of Timolol, Biotin and Coniine are the WWTPs and the communities because the concentration of these PPCPs increase at points just downstream these key sampling points (see Graph 3-1and Annex 6 for detailed results).

The close proximity of the Kat River dam to the Seymour Waste Water Treatment Plant (WWTP) is of major concern. The Kat River dam is located less than a kilometre downstream the Seymour WWTP. The results obtained from the Kat River dam water analysis showed that the dam is contaminated with PPCPs from the WWTP just upstream. This is a major concern because the Kat River dam supplies drinking water for both Seymour and Fort Beaufort towns. Drinking water for Seymour is directly abstracted from the dam, whereas the Fort Beaufort water is released into a river channel and abstracted from a weir near town, where it is treated and distributed to the consumers in town.

4 CHAPTER FOUR

GENERAL DISCUSSION AND RECOMMENDATIONS

4.0 Introduction

In South Africa, measures or control programmes have been put in place for both water and food pesticide contamination. However, as new pesticides are being continuously introduced, not all pesticides and Pharmaceuticals and Personal Care Products (PPCPs) have well established Maximum Residue Limits (MRLs). The challenge is therefore determining the extent of potential environmental contamination and potential human health effects of these pesticides. Even though a single pesticide can be considered safe at a particular concentration level, it is still possible for the pesticide to reach toxic levels in the environment due to accumulation. The accumulative effect and continuous exposure to low concentration levels also have a huge impact to individuals exposed to these low concentration levels of pesticides and PPCPs. In addition non-point source pollution, which is unpredictable, may result in contamination of crops grown by small scale farmers or which are not monitored for pesticide residues, thereby exposing many families who are eating the crops to pesticide contamination. Fenske (2000) carried a study in which he explored the cumulative dietary pesticide intake by children. He concluded that up to 56% of children exceeded the acceptable chronic dietary dose of spirodiclofen and imidacloprid pesticides (Fenske, 2000).

In South Africa there has been on-going research on the impact of pesticides in the environment and their effect on human health, as a result of increased use of pesticides in agriculture in order to maximise crop yields. The research has highlighted the possible effects of pesticide contaminants in environment on human health (Drouillet-Pinard et al., 2010) and the need to continuously monitor the environmental contamination of pesticides and PPCPs. However the lack of laboratory equipment, which is sensitive enough to detect low levels of contaminants to be used for routine screening and quantification of pesticides and PPCPs, has been a major concern. Although pesticides have been detected in waste water, there is still lack of knowledge of background environmental levels, as these levels can help in the impact and health assessments. It is also very important to note that the health risks associated with pesticides or PPCPs are not limited to occupational exposure, but end-user exposure as well. MRLs were set to protect both those who are consuming the products and those who are

importing the products. South African MRLs are in the same range as those stipulated by the European Union. On paper, this is very important as it allows continuous trade and protection of the health of South African citizens; however the challenge is in the control measures for making sure these residue limits are practised and implemented.

4.1 Discussion

The identification of possible pesticide contaminants and their relative concentration at various sampling points on the Kat River water was achieved by the use of the direct injection method. The challenge of laboratory equipment to detect low concentration levels of peptides in the Kat River water was nullified by the ability of the new 5600 ABSCIEX Triple TOF coupled to the 1260 Agilent auto sampler to detect pesticides residues at as low concentrations as parts per million(ppm). The concentration levels of the pesticides are determined based on the intensity of their peaks. The sources of the detected pesticides were identified by monitoring the spray regime of farmers around each sampling point and determining if the detected pesticides are part of the spray regime. The data obtained from the analysis of pesticides in Kat River confirm that the agricultural activities on the Kat River banks have a huge impact on downstream water health and food security due to pesticide river contamination. It was observed that some of the pesticides from the spray regimes are persistent and therefore contaminate the river downstream. It was also deduced that some farmers were using the same spray regime, a case where pesticide concentration increased at successive points downstream.

The data obtained from the analysis of pesticide from fruits and vegetables confirmed that the agricultural activities by commercial farmers have a direct impact on the health and safety of food crops grown by small scale farmers whose products may not be monitored for Maximum residue limits before consumption. Pesticides from the spray regimes detected in the Kat River were also detected in vegetables (Spinach, Cabbage and tomatoes). Therefore, the sources of the detected pesticides in fruits and vegetables are the farms around the sampling points on Kat River at which the pesticides were detected. The data from the analysis of fruits also confirmed pesticide contamination of fruits (Lemons and oranges). The sources of the pesticide contaminants are the commercial farmers who spray on their orchards to protect their crops to maximize crop yield. However, while protecting their own crops by using pesticides, their own crops are also exposed to pesticide contamination. This was confirmed as results showed that some of the pesticides from their spray regime were also

detected on oranges and lemons from their farms. This means that the pesticides are degrading slowly, and therefore are contaminating their own crops, the crops by small scale farmers and the river downstream. We therefore conclude that pesticide use for agricultural activities on the Kat River have a huge environmental impact on downstream water, health, security and huge effects on human health as people might be exposed to the food crops contaminated with pesticide residues.

The data obtained from the analysis of PPCPs confirmed that Kat River is also prone to PPCPs contamination. Results showed that there is huge influx of PPCPS into the Kat River downstream of waste water treatment plants. The Kat River is not only used for irrigation purposes but for supplying drinking water as well. The level of PPCP contamination on the Kat River Dam has a huge impact on human health as this may expose people to PPCPs through drinking water not monitored for PPCPs maximum residue limits. PPCPs exposure has a direct impact on human health as people might develop resistance to antibiotics.

4.2 Recommendations

- We recommend that water quality monitoring programs be put in place for both Kat River water and drinking water for communities depending on the Kat River Dam to help reduce the exposure of Pesticide and Pharmaceuticals and Personal Care Product to people.
- We also recommend that the fruits and vegetables of farmers be monitored for Maximum residue limits to help reduce the impact on their health due to exposure to pesticide residues.
- We also recommend that the Waste Water Treatment Plants (WWTPs) be monitored regularly to make sure they are effectively degrading the influent fed to before releasing it to the environment. On this writing, both the WWTPs (Seymour and Fort Beaufort) are not operating to full potential as some pipes are leaking. We also recommend that the location of Seymour WWTP be re-considered as it a direct PPCP contaminant of the Kat River Dam.

4.3 Conclusion

This is the first report on the identification of agricultural and industrial contaminants (pesticide and Pharmaceuticals and Personal Care Products) on the Kat River, Eastern Cape

Province particularly with regard to their possible effect on agricultural food products and human settlements found on/or near the Kat River banks. The results reported here in this thesis indicate that there is contamination in the Kat River water due to both pesticides and PPCPs. The results also indicate that the food products are also contaminated and hence both the Kat River agricultural produce and its water need to be closely monitored for both pesticide and PPCPs contaminants.

4.4 Future studies

As part of our future studies, we would like to explore the following avenues:

1. To further investigate the quantitative levels of pesticides and PPCPs in Kat river water to determine if the concentration levels of the detected pesticides are below the reported Maximum Residues Limits (MRL) (see Annex 2 for South African MRL for pesticides on food crops).This is going to be achieved by buying the standards of the detected pesticide and spike them with the samples and determine the percentage recoveries.
2. To further investigate the concentration levels of pesticide residues in agricultural products from Kat River, both commercial and small scale produces. This will help to determine the level of exposure of people to pesticide residues. This will also allow us to give feedback to the small scale farmers whose food crops may not be monitored for pesticide residues before consumption.
3. To further investigate the concentration level of Pharmaceuticals and Personal Care Products (PPCPs) and pesticides in drinking water. This is going to be achieved by buying the standards of identified PPCPs and pesticides. Drinking water samples will be collected from households in communities depended on Kat River dam for drinking water. The samples will be run against the standards on LC/MS.
4. River water contamination is not only limited to PPCPs and pesticides. We also look forward to identify and determine the level of microbial contamination in the Kat River as it is part of the farmers' agricultural practices to monitor the level of bacterial contamination in the water used for irrigation, especially *Escherichia coli.*
5. We also want to extend this project to other rivers in the Eastern Cape Province. This is going to help not only the victims of these contaminants but also the country as whole.

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Annextures

Annex 1: Banned Pesticides in South Africa

Aldrin (HHDN) Withdrawn in 1992.

Arsenic. All uses of any inorganic arsenic containing compound on plant material (except on citrus) were banned in 1983. In 1983 it was also totally prohibited as a stock remedy.

Atrazine. Withdrawn from use on heavy clay soils (Springbok Flats) in 1977. The industrial use withdrawn on 31 March 1995.

Azinphos-ethyl. Withdrawn as an agricultural remedy in 1997.

BHC (mixture of various isomers). Banned in 1983.

Binapacryl. All registrations expired in 1988.

Camphechlor (CLC). Withdrawn as an agricultural remedy in 1970 and as a stock remedy in 1985.

Chlordane. In 1993 use restricted to stem treatment of citrus and vineyards and the treatment of structures by pest control operators. Withdrawn as an agricultural remedy in 2000.

Chlordane. Banned in 2005.

Chlordimeform. Withdrawn as an agricultural remedy in 1978.

Chlorobenzilate. Withdrawn as an agricultural remedy in 1978.

2,4-D (dimethylamine salt). In 1991 aerial application in Natal was banned and it has been totally prohibited in parts of the magisterial districts of Camperdown, Pietermaritzburg and Richmond.

2,4-D esters. In 1980 it was withdrawn from all agricultural uses in the Western Cape and prohibited in 1991 in Natal.

2,4-DB (sodium salt). In 1991 aerial application in Natal was banned and it has been totally prohibited in parts of the magisterial districts of Camperdown, Pietermaritzburg and Richmond.

Dicamba. In 1991 aerial application in Natal was banned and it has been totally prohibited in parts of the magisterial districts of Camperdown, Pietermaritzburg and Richmond.

DDT. Banned in 1983 except for the control of malaria by the Government.

Dibromochloropropane. Withdrawn in 1984.

Dieldrin. Banned in 1983.

Dinoseb. All registrations as an agricultural remedy expired in March 1995.

DNOC - Withdrawn as an agricultural remedy in 2001

Endosulfan. Registration on fodder crops was suspended in 1970.

Endrin (Nendrin). Withdrawn in 1980.

Gamma-BHC (lindane). All stock remedy registrations were withdrawn in 1971. All registrations under the Department of Agriculture to be withdrawn by April 2009.

Heptachlor. Registration was withdrawn in 1976.

Kepone. In 1971 a decision was taken not to allow this product in South Africa.

Leptophos. Registration was suspended in 1980.

MCPA (dimethylamine salt). In 1991 aerial application in Natal was banned and it has been totally prohibited in parts of the magisterial districts of Camperdown, Pietermaritzburg and Richmond.

MCPA (potassium salt). In 1991 aerial application in Natal was banned.

MCPB (sodium salt). In 1991 aerial application in Natal was banned.

Mercury compounds. It was withdrawn from all agricultural uses in 1974. In 1983 the use of all mercury compounds on seed, bulbs, tubers, stems or any other plant material was banned.

Methyl bromide. All small packages (680 g) were withdrawn in December 1995.

Monocrotophos. The use as leaf application on citrus, cutworm control in carrots and use on tomatoes were withdrawn in 1997. On 25 February 2005 all products containing monocrotophos approved by the Registrar was banned.

Monocrotophos. Banned in 2005.

Nicotine. It was withdrawn from use as a stock remedy in 1971.

Parathion. Only certain uses allowed from June 1993.

Phosphorus containing formulations. In 1979 all formulations containing phosphorus were withdrawn.

Propham. Withdrawn as an agricultural remedy in 1997.

2,4,5-T. All registrations expired in 1989.

Triclopyr. In 1991 aerial application in Natal was banned.

TDE. Withdrawn as an agricultural remedy in 1970.

Annex 2: Pesticides registered in South Africa, including information on chemical classification, application use and relevant crops as indicated by South African MRL levels (South African Department of Health (DOH), 2005; PAN, 2010).

Chemical	Pesticide notes	South African MRL (mg kg^{-1}) and associated crops
Propyzamide	Amide herbicide	0.02 - 0.1: Apples, apricots, cherries, grapes, peaches, pears and plums
Diphenylamine	Amine fungicide, insecticide and plant growth regulator	10: Apples and pears
Boscalid	Anilide fungicide	5: Grapes
Fenhexamid	Anilide fungicide	5: Grapes
Clodinafop-propargyl	Aryloxyphenoxy propionic acid	0.05: Wheat
Fluazifop-P-butyl	Aryloxyphenoxy propionic acid herbicide	0.01 - 0.2: Apples, apricots, beans, carrots, coffee, grapes, nuts, peaches, pears, plums, potatoes, quinces, soya beans and sugar cane
Haloxyfop-R	Aryloxyphenoxy propionic acid herbicide	0.05: Apples, apricots, citrus, grapes, peaches, pears, pineapples and plums; 0.1 - 0.5: Beans, beetroot, cotton seed, dry beans, peas, soya beans and sugar cane; 1 - 2: Groundnuts and lucerne
Propaquizafop	Aryloxyphenoxy propionic acid herbicide	0: Milk; 0.05 - 0.2: peas, cucurbits and clover
Bromuconazole	Azole fungicide	0.02 - 0.2: Apples, barley and wheat
Cyproconazole	Azole fungicide	0.02 - 0.1: Apples, barley, coffee, dry beans, grapes, pears, peas and wheat. 0.2 - 1: Cucurbits and oats
Difenoconazole	Azole fungicide	0.05 - 0.5: Apples, beans, citrus, grapes, groundnuts, pears, potatoes and tomatoes.
Fenbuconazole	Azole fungicide	0.05 - 0.1: Apples, barley, pears, plums and wheat; 1: Apricots and peaches
Flusilazole	Azole fungicide	0.01 - 0.1: Apples, barley, dry beans, grapes, groundnuts, mangoes, pears, peas and wheat
Flutriafol	Azole fungicide	0.05 - 0.1: Apples, barley, dry beans, peaches, pears, soya beans and wheat
Hexaconazole	Azole fungicide	0.01 - 0.05: Cucurbits, dry beans and mangoes; 0.1 - 1: Apples, grapes, peaches, pears, pineapples and pears
Imazalil	Azole fungicide	0.5: Cucurbits; 5: Citrus and musk melons
Myclobutanil	Azole fungicide	0.05 - 0.5 Cucurbits, dry beans, grapes and pears

Chemical	Pesticide notes	South African MRL (mg kg^{-1}) and associated crops
Penconazole	Azole fungicide	0.02 - 0.2: Apples, cucurbits, grapes, pears and peas
Prochloraz	Azole fungicide	0.1 - 0.2: Barley, mushrooms, potatoes and wheat; 2- 10: Avocadoes, bananas, citrus, ginger and mangoes
Propiconazole	Azole fungicide	0.05 - 0.5: Bananas, barley, grapes, groundnuts, maize, peaches, nuts and wheat
Tebuconazole	Azole fungicide	0.02 - 0.1: Barley, beans, citrus, groundnuts, mangoes, oats, onions, potatoes, soya beans, tomatoes and wheat; 2 - 5: grapes
Tetraconazole	Azole fungicide	0.5: Grapes
Triadimefon	Azole fungicide	0.05 - 0.5: Apples, bananas, barley, cucurbits, mangoes, oats, peas and wheat; 2: Grapes
Triadimenol	Azole fungicide	0.05 - 0.5: cucurbits, peas, soya beans and apples; 1: Grapes
Paclobutrazol	Azole plant growth regulator	0.05: Avocadoes, litchis, nuts, mangoes, peaches and plums
Zoxamide	Benzamide fungicide	0.05: Potatoes and 2: grapes
Benomyl	Benzimidazole fungicide	0.05 - 0.1: Maize, groundnuts, pears, sugar cane and wheat. 1 - 3: Apples, apricots, avocados, bananas, grapes, peaches, pears, peppers, plums and tomatoes. 5: Citrus and mangoes.
Carbendazim	Benzimidazole fungicide	0.01 - 0.1: Avocadoes, chicory, dry beans, groundnuts, mangoes, maize, oats and potatoes. 0.2 - 1: Grapes, peas and tomatoes. 3 - 5: Apples, citrus and pears
Thiabendazole	Benzimidazole fungicide	1 - 10: Apples, avocadoes, bananas, citrus, mushroom, musk melons, pears, pineapples and potatoes
Novaluron	Benzolurea herbicide	0.01 - 0.05: Apples, cotton seed, canned peaches, pears and tomatoes
Lufenuron	Benzolurea insecticides	0.02 - 0.1: Tomatoes and cabbage
Acibenzolar-S-methyl	Benzothiadiazole plant activator and fungicide	0.2 - 0.5: Tomatoes and mangoes
Diflubenzuron	Benzoyl urea	0.01: Potatoes, 0.1: mushrooms, 1: apples and pears.
Flufenoxuron	Benzoyl urea insecticide	0.05: Apples and pears
Teflubenzuron	Benzoyl urea insecticide	0.02 - 0.5: Citrus and litchis

Chemical	Pesticide notes	South African MRL (mg kg^{-1}) and associated crops
Triflumuron	Benzoyl urea insecticide	0.1 - 0.5: Chicken fat, citrus, litchis, mangoes and peaches; 2: Apples and pears
Thiophanate-methyl	Bezimidazole precursor fungicide	0.1: Barley, groundnuts and wheat; 3 - 5: Apples, citrus and pears
Dicamba	Benzoic acid herbicide	0.1 - 0.2: Maize, sorghum, sugar cane and wheat.
Diquat dibromide	Bipyridylium desiccant and herbicide	0.05: Potatoes and 0.5: sunflower seed
Paraquat dichloride	Bipyridylium herbicide	0.02 - 0.5: Cotton seed, maize, sugar cane
Emamectin, benzoate	Botanical insecticide	0.01: Tomatoes
Pyrethrins	Botanical insecticide	1 - 2: Apples, apricots, beans, broccoli, Brussels sprouts, cabbage, cauliflower, cereal grains, citrus, cotton seed, cucurbits, dried fruit, dried nuts, dried vegetables, grapes, groundnuts, guavas, lettuce, oil seeds, peaches, plums, sunflower seed and tomatoes
Gibberellins	Botanical plant growth regulator	0.05- 0.2Apples, citrus and grapes
Iprovalicarb	Carbamate fungicide	0.05 - 0.5: Grapes, potatoes and tomatoes
Maneb	Carbamate fungicide	0.01: all foodstuffs except cereal grains and grapes. 0.1: cereal grains. 180: grapes
Oxycarboxin	Carbamate fungicide	0.5: Beans
Propamocarb hydrochloride	Carbamate fungicide	0.5: Potatoes and 2: cucumbers
Thiram	Carbamate fungicide	3 - 5: Apples, apricots, grapes, peaches, pears and plums
Carbosulfan	Carbamate insecticide	0.05 - 0.2: Grapes and maize
Formetanate	Carbamate insecticide	0.02 - 0.5: Apples, citrus, grapes and peaches
Methiocarb	Carbamate insecticide	0.1 - 0.2: Apples, apricots, citrus, grapes, pears and plums
Methomyl	Carbamate insecticide	0.02 - 0.2: Beans, broccoli, Brussels sprouts, cabbage, cauliflower, citrus, maize, peaches, potatoes, sorghum, sunflower seed, tomatoes and wheat

Chemical	Pesticide notes	South African MRL (mg kg^{-1}) and associated crops
Pirimicarb	Carbamate insecticide	0.05 - 0.5: Apples, broccoli, Brussels sprouts, cabbage, cauliflower, citrus, cotton seed, groundnuts, oats, peaches, nuts, potatoes, sorghum and wheat
Propoxur	Carbamate insecticide	0.05: grapes
Thiodicarb	Carbamate insecticide	0.1 - 0.5 Cotton seed and maize
Carbofuran	Carbamate insecticide and nematicide	0.05 - 0.5: Broccoli, Brussels sprouts, cabbage, cauliflower, cotton seed, maize, potatoes, sorghum, sugar cane, sunflower seed and wheat.
Carbaryl	Carbamate insecticide, nematicide and plant growth regulator	0.1 - 0.5: Cactus pears, castor oil seed, cottonseed, maize, meat, eggs, milk and poultry. 2.5: Apples, apricots, beans, grapes, pears, sorghum and wheat
Aldicarb	Carbamate pesticide	0.05: cottonseed, nuts, maize and pineapples. 0.2-0.5: Bananas, citrus, coffee, grapes, groundnuts, sweet potatoes and tomatoes. 1 -2: Fodder (hay), potatoes and hops (dry).
Bendiocarb	Carbamate pesticide	0.1 - 0.2: Maize and sorghum
EPTC	Carbamate pesticide	Beans, maize, potatoes, sugar cane, sunflower seed, sweet corn and sweet potatoes
Dichlorophene	Chlorinated phenol fungicide, herbicide, microbiocide	Pineapples, potatoes and tomatoes.
Alachlor	Chloroacetanilide	0.05 - 0.1: Broccoli, Brussels sprouts, cabbage, groundnuts, maize, pineapples, potatoes, soya beans, sugar cane and sunflower seed
Acetochlor	Chloroacetanilide herbicide	0.02- 0.05: Cotton seed, groundnuts, maize, sorghum and sugar cane
Metazachlor	Chloroacetanilide herbicide	0.05 - 0.1: Cabbage, dry beans, groundnuts, maize, potatoes, sugar cane, sunflower seed and sweet corn
Metolachlor	Chloroacetanilide herbicide	0.05: Beans, cotton seed, dry beans, groundnuts, maize, potatoes, sorghum, soya beans, sugar cane and sunflower seed
Propachlor	Chloroacetanilide herbicide	0.1 - 0.2: Maize, onions and sorghum

Chemical	Pesticide notes	South African MRL (mg kg^{-1}) and associated crops
Diclofop-methyl	Chlorophenoxy acid or ester herbicide	0.05: Wheat
MCPA and its salts	Chlorophenoxy acid or ester herbicide	0.1: Barley, maize, potatoes, rye, sorghum, sugar cane and wheat
Triclopyr	Chloropyridinyl herbicide	0.1: Citrus
Cycloxydim	Cyclohexenone derivative herbicide	0.5: Beans, cottonseed, cucurbits, dry beans, grapes, groundnuts, onions, soya beans and tomatoes.
Tralkoxydim	Cyclohexenone derivative herbicide	0.05: Barley and wheat
Dimethipin	Defoliant and plant growth regulator	0.1: Cotton seed
Tebufenozide	Diacylhydrazine insecticide	1: Apples and pears
Iprodione	Dicarboximide fungicide	0.05 - 0.5: ginger, onions and canned peaches; 1 - 5: Apricots, apples, citrus, grapes, kiwifruit, peaches, pears, plums, raspberries, strawberries and tomatoes
Vinclozolin	Dicarboximide non-systemic general use pesticide and fungicide	1 - 3: Strawberries and grapes
Pendimethalin	Dinitroaniline herbicide	0.05: Potatoes
Trifluralin	Dinitroaniline herbicide	0.05: Cabbage, chillies, cowpeas, dry beans, groundnuts, kidney beans, soya beans, sunflower seeds and tomatoes; 1: Carrots
Dinocap	Dinitrophenol derivative fungicide and insecticide	1: Apples, broccoli, Brussels sprouts, cabbage, cauliflowers, cucurbits, grapes, peaches, pears and peas
Fomesafen	Diphenyl ether herbicide	0.05: Dry beans, groundnuts and soya beans
Oxyfluorfen	Diphenyl ether herbicide	0.05: Citrus and garlic
Zineb	Dithiocarbamate fungicide	0.05 - 0.5: Groundnuts, onions and potatoes; 3: Apples, apricots, bananas, beans, boysenberries, broccoli, Brussels sprouts, cabbage, cauliflower, citrus, cucurbits, grapes, guavas, mangoes, olives, papayas, peaches, pears, peppers, plums, quinces, tomatoes and youngberries
Furfural	Fumigant	Carrots, lettuce, onions, potatoes and sugar cane

Chemical	Pesticide notes	South African MRL (mg kg^{-1}) and associated crops
Cymoxanil	Fungicide	0.01 - 0.2: Grapes, potatoes and tomatoes
Dithianon	Fungicide	2: Apples, apricots, peaches, pears and plums
Epoxiconazole	Fungicide	0.01 - < 0.05: Maize and barley
Famoxadone	Fungicide	0.01 - 0.02: Potatoes, 0.2: tomatoes, 1: grapes
Fludioxonil	Fungicide	0.5: Grapes
Fosetyl-Al	Fungicide	5 - 50: Avocadoes, boysenberries, citrus, cucumber, grapes, pineapples, potatoes and youngberries
Guazatine	Fungicide	2.5: Tomatoes and 5: Citrus
Spiroxamine	Fungicide	0.05: Barley and wheat; 0.1: peas and 1: grapes
Dodine	Guanidine fungicide and microbiocide	1: Apples, pears and quinces
Methyl bromide	Halogenated organic fumigant, herbicide, insecticide and nematicide	10- 100: Cereal grains, dried fruit, dried legumes, processed grain products and groundnuts
Cyhexatin	Heavy metal, organotin insecticide	2: Apples, citrus, peaches, pears, plums and tomatoes. 150: Hops (dry).
Fenbutatin-oxide	Heavy metal, organotin insecticide	0.2 - 2: Apples, beans, citrus, peaches, pears, peppers and tomatoes
2,4-D	Herbicide	0.5 - 2: Barley, citrus, maize, potatoes, rye, sorghum, sugar cane and wheat
Fluorochloridone	Herbicide	0.02 - 0.05: Apples, carrots, grapes, nectarines, pears, plums, potatoes and sunflower seed
Mesotrione	Herbicide	0.01: Maize
Sulcotrione	Herbicide	0.05: Maize and sugar cane
Ioxynil	Hydroxybenzonitrile herbicide	0.05: Sugar cane
Bromoxynil phenol	Hydroxybenzonitrile insecticide	0.1: Barley, maize, oats, sorghum, sugar cane and wheat
Imazapyr	Imidazolinone herbicide	0.05: Dry beans, groundnuts and soya beans
Magnesium phosphide	Inorganic fumigant and rodenticide	0.01: all foodstuffs except cereal grains and grapes. 0.1: cereal grains. 180: grapes

Chemical	Pesticide notes	South African MRL (mg kg^{-1}) and associated crops
Phosphoric acid	Inorganic fungicide, herbicide, antimicrobial and pH adjuster	25 - 50: Grapes and citrus
Calcium arsenate	Inorganic heavy metal herbicide, insecticide and rodenticide	0.2: Citrus
Sulphur	Inorganic herbicide and insecticide	50 - 55: Apples, apricots, avocadoes, bananas, beans, boysenberries, citrus, cucurbits, grapes, litchis (pulp), mangoes, papaya, peaches, pears, peas, peppers, plums, tomatoes and youngberries; 1 000: litchis peel
Aluminum phosphide	Inorganic phosphide fumigant	0.01: all foodstuffs except cereal grains and grapes. 0.1: cereal grains. 180: grapes
Propineb	Inorganic -zink carbamate antimicrobial and fungicide	0.5: Groundnuts and potatoes; 3: Boysenberries, grapes, tomatoes and youngberries
Mancozeb	Inorganic-zinc carbamate fungicide	0.01: all foodstuffs except cereal grains and grapes. 0.1: cereal grains. 180: grapes
Metiram	Inorganic-zinc carbamate fungicide	0.5: Potatoes; 3: Apples, apricots, beans, grapes, peaches, pears, plums and tomatoes
Buprofezin	Insect growth regulator	0.05: Avocadoes and peaches
Bromopropylate	Insecticide	0.2 - 3: Bananas, citrus, cotton seed and grapes
Etoxazole	Insecticide	0.1 - 0.2: Apples, pears and tomatoes
Fenazaquin	Insecticide	0.05 - 0.5: Apples, citrus, pears and tomatoes
Indoxacarb, S-isomer	Insecticide	0.01 - 0.05: Potatoes, cauliflower; 0.02 -0.2: Tomatoes, beans, peaches and peas; 1: Apples, cabbage, broccoli, Brussels sprouts and pears
Propargite	Insecticide	0.05 - 0.5: Cotton seed and pears; 2 - 3: Apples, citrus, peaches, strawberries and tomatoes
Tetradifon	Insecticide	0.05: Cotton seed; 5 - 8: Apples, apricots, citrus, cotton seed, peaches, pears, plums and dry tea
Triforine	Insecticide and fungicide	0.1 - 0.5: Cucurbits and peas; 1 - 2: Apples, beans, peaches and plums
Spirodiclofen	Keto-enol insecticide	0.01: Peaches

Chemical	Pesticide notes	South African MRL (mg kg^{-1}) and associated crops
Ametryne	Methylthiotriazine herbicide	0.05 - 0.2: Bananas, maize, pineapples and sugar cane
Milbemectin	Microbial insecticide	0.01: Apples and tomatoes
Spinosad	Microbial insecticide	0.01 - 0.5: Apples, apricots, beans, citrus, cabbage, cucurbits, grapes, guavas, mangoes, olives, peaches, pears, peas, plums, potatoes and tomatoes
Dimethomorph	Morpholine fungicide	0.01: Potatoes, 0.1: tomatoes and 5: grapes
Tridemorph	Morpholine fungicide	0.1 - 0.2: Cucurbits and peas
Copper and its salts	Multiple forms and uses	1: Potatoes and nuts. 20: Apples, apricots, avocados, beans, boysenberries, broccoli, Brussels sprouts, cabbage, cauliflower, celery, cherries, citrus, coffee, cucurbits, granadillas, grapes, guavas, lettuce, mangoes, olives, peaches, pears, peppers, plums, strawberries, tomatoes and youngberries.
Acetamiprid	Neonicotinoid insecticide	0.2 - 0.50: Barley, canola, citrus, oats, cotton seed, tomatoes and wheat
Imidacloprid	Neonicotinoid insecticide	0.05 - 0.5: Apples, citrus, cotton seed, cucurbits, grapes, maize, sorghum, sunflower seed, tomatoes and wheat
Thiacloprid	Neonicotinoid insecticide	0.1: Peaches and 1: apples
Thiamethoxam	Neonicotinoid insecticide and fungicide	0.02 - 0.05: Apples and cotton seed
Cartap monohydrochloride	Nereistoxin insecticide	5: Onions, 10: tomatoes and 150: cabbage
MSMA	Organoarsenic defoliant and herbicide	0.05: Sugar cane
Dicofol	Organochlorine insecticide	Apples, apricots, bananas, beans, broccoli, Brussels sprouts, cabbage, cauliflower, cherries, citrus, cotton seed, cucurbits, granadillas, peaches, pears, peas, peppers, plums, quinces and tomatoes.

Chemical	Pesticide notes	South African MRL (mg kg^{-1}) and associated crops
Endosulfan	Organochlorine insecticide	0.05: Granadillas, nuts, pineapples and potatoes; 0.1 - 1: Apples, apricots, Boysenberries, broccoli, Brussels sprouts, cabbage, cauliflower, cherries, citrus, coffee, cotton seed, cucurbits, grapes, groundnuts, maize, onions, paprika, peaches, pears, peas, plums, quinces, sorghum, sugar cane, sunflower, tomatoes, wheat and youngberries; 20: Hops (dry)
Lindane	Organochlorine insecticide and rodenticide	0.01 - 0.02: Milk, cottonseed, onions, potatoes and sweet potatoes; 1: Apples, apricots, beans, broccoli, Brussels sprouts, cabbage, cauliflower, peaches, pears, plums.
Fenthion	Organophosphate acvicide and insecticide	0.1 - 1: Apples, apricots, coffee, cucurbits, grapes, guavas, kiwifruit, mangoes, peaches, pears, plums and quinces.
Acephate	Organophosphate insecticide	1 - 3: Apples, broccoli, Brussels sprouts, cabbage, cauliflower, grapes, peaches, pears, plums, potatoes and tomatoes
Azinphos-methyl	Organophosphate insecticide	0.05: Cottonseed, olives and potatoes. 0.04: Apples and pears. 0.1 - 0.2: Apricots, citrus, peaches and plums.
Cadusafos	Organophosphate insecticide	0.02 - 0.05: Bananas, citrus and potatoes
Chlorpyrifos-methyl	Organophosphate insecticide	8: Cereal grains
Malathion / Mercaptothion	Organophosphate insecticide	0.05: maize, peas, onions, sorghum and sugar cane; 1 - 8: Apples apricots, avocadoes, bananas, beans, broccoli, Brussels sprouts, cabbage, cauliflower, cereal grains, citrus clover, cotton seed, cucurbits, dried fruits, dried nuts, granadillas, grapes, groundnuts, guavas, litchis, mangoes, mushrooms, oil seeds, papayas, peaches, pears, peppers, pineapples, plums, quinces, sunflower seed and tomatoes

Chemical	Pesticide notes	South African MRL (mg kg^{-1}) and associated crops
Oxydemeton-methyl	Organophosphate insecticide	0.1 - 0.4: Apples, apricots, beans, broccoli, Brussels sprouts, cabbage, cauliflower, citrus, cotton seed, cucurbits, aubergine, groundnuts, maize, onions, peaches, pears, peas, peppers, plums, potatoes, Rooibos, sorghum, tomatoes and wheat.
Parathion	Organophosphate insecticide	0.05 - 0.5: Barley, beans, beetroot, broccoli, Brussels sprouts, cabbage, cactus pears, carrots, castor-oil seed, cauliflower, citrus, coffee, cotton seed, cucurbits, aubergine, groundnuts, mangoes, onions, peas, peppers, quinces, sorghum, spinach, sweet potatoes, tomatoes, turnips and wheat
Phenthoate	Organophosphate insecticide	0.1 - 0.2: Mangoes, onions and potatoes; 1: Broccoli, Brussels sprouts, cabbage, cauliflower and citrus
Phoxim	Organophosphate insecticide	0.2: Cereal grains and groundnuts
Pirimiphos-methyl	Organophosphate insecticide	3 - 10: Groundnuts, maize, sorghum, soya beans, stored wheat and sunflower seed
Procymidone	Organophosphate insecticide	0.05 - 0.5: Citrus, groundnuts, pears and potatoes; 1 - 10: Beans, grapes, peaches, plums and tomatoes
Prothiofos	Organophosphate insecticide	0.05: Apples, apricots, citrus, mangoes, pears and plums; 1: Grapes and guavas
Temephos	Organophosphate insecticide	1: Citrus
Trichlorfon	Organophosphate insecticide	0.05 - 0.2: Apples, apricots, broccoli, Brussels sprouts, cabbage, cauliflower, citrus, coffee, cucurbits, granadillas, grapes, guavas, litchis, maize, peaches, plums, quinces and sweet potatoes; 1: Beans and tomatoes

Chemical	Pesticide notes	South African MRL (mg kg^{-1}) and associated crops
Demeton-S-methyl (mixture)†	Organophosphate insecticide	0.1 - 0.5: Apples, apricots, barley, beans, broccoli, Brussels sprouts, cabbage, cauliflower, citrus, cotton seed, eggplant, groundnuts, maize, olives, onions, peaches, pears, peas, peppers, plums, potatoes, Rooibos, sorghum ,tomatoes and wheat
Diazinon	Organophosphate insecticide	0.02: Milk, 0.2 - 0.7: apples, apricots, beans, broccoli, brussels sprouts, cabbage, meat, cauliflower, mushrooms, peaches, pears, pineapples, plums and tomatoes
Dimethoate	Organophosphate insecticide	Apples, barley, beans, broccoli, brussels sprouts, cabbage, cauliflower, citrus, cotton seed, cucurbits, grapes, groundnuts, peaches, pears, pineapples, plums, potatoes, sorghum, strawberries and wheat
Methamidophos	Organophosphate insecticide	0.05 - 0.5: Canola, citrus, potatoes and tomatoes; 1: Apples, apricots, broccoli, brussels sprouts, cabbage, mangoes, peaches, pears and plums
Methidathion	Organophosphate insecticide	0.02 - 0.3: Apples, apricots, cactus pears, cherries, grapes, peaches, pears, plums and potatoes; 2: Citrus
Mevinphos	Organophosphate insecticide	0.05: Potatoes; 0.1 - 0.2: Beans, broccoli, brussels sprouts, cabbage, cauliflower, citrus, cucurbits, grapes, lettuce, peas, peppers, spinach, tomatoes and wheat
Omethoate	Organophosphate insecticide	0.05 - 0.5: Barley, cotton seed, oats and onions; 1 - 1.5: Apples, grapes, pears, peas and wheat
Phosmet	Organophosphate insecticide	2 - 5: Apples and pears
Profenofos	Organophosphate insecticide	0.05: Onions and potatoes; 0.5 - 1: Brussels sprouts, cabbage, cauliflower, citrus and tomatoes

Chemical	Pesticide notes	South African MRL (mg kg^{-1}) and associated crops
Chlorpyrifos	Organophosphate insecticide and nematicide	0.05 - 1: Apples, apricots, bananas, broccoli, Brussels sprouts, cabbage, carrots, cauliflower, citrus, grapes, lettuce, mangoes, maize, wheat, peaches, pears, plums, potatoes and tomatoes.
Disulfoton	Organophosphate insecticide and nematicide	0.05 - 0.5: Cabbage, cauliflower, coffee, cotton seed, onions, potatoes, tomatoes and wheat
Ethoprop	Organophosphate insecticide and nematicide	0.01: Potatoes and 0.05: citrus
Fenamiphos	Organophosphate insecticide and nematicide	0.01 - 0.2: Bananas, citrus, cotton seed, ginger, grapes, groundnuts, guavas, litchis, onions, papaya, peaches, peas, nuts, pineapples, potatoes and tomatoes
Methyl parathion	Organophosphate insecticide and nematicide	0.05: Coffee and 1: citrus
Phorate	Organophosphate insecticide and nematicide	0.05: Apples, broccoli, Brussels sprouts, cabbage, cauliflower, cotton seed, maize, onions, potatoes and wheat
Terbufos	Organophosphate insecticide and nematicide	0.05 - 0.1: Citrus, dry beans, groundnuts, maize, potatoes, sorghum and sunflower seed
Fosthiazate	Organophosphate nematicide	0.05- 0.1: Bananas, citrus and potatoes
Ethephon	Organophosphate plant growth regulator	0.05: Maize and sugar cane. 1 - 5: Apples, cherries, citrus, cotton seed, grapes, peaches, pineapples, plums and wheat
Ortho-phenylphenol	Phenol antimicrobial	10: Citrus
Glyphosate and its salts	Phosphonoglycine herbicide	0.5: Sugar cane and 2: Maize
1-Naphthaleneacetic acid, methyl ester	Plant growth regulator	1: Apples and pears
Chlorfenapyr	Pyrazole insecticide	0.01 - 0.5: Apples, citrus, grapes, nectarines, pears, plums, potatoes and tomatoes.
Fipronil	Pyrazole insecticide	0.01 - 0.05Broccoli, cabbage, cauliflower, citrus and mangoes
Pyraflufen-ethyl	Pyrazolylphenyl herbicide	0.01: Barley and wheat

Chemical	Pesticide notes	South African MRL (mg kg^{-1}) and associated crops
Bioresmethrin	Pyrethroid insecticide	0.05: Groundnuts. 0.1 - 1: Apples, apricots, beans, peaches, pears and plums.
Cyfluthrin	Pyrethroid insecticide	0.05: Cottonseed. 0.1 - 0.2: Apples, beans, broccoli, Brussels sprouts, cabbage, cauliflower, grapes, maize, pears, peas, sorghum and tomatoes. 1: Wheat
Cyfluthrin, beta	Pyrethroid insecticide	0.05 - 0.2: Apples, beans, broccoli, Brussels sprouts, cabbage, canola, cauliflower, cotton seed, grapes, nuts, maize, peaches, pears, peas, potatoes, sorghum, tomatoes and wheat.
Cyhalothrin, gamma	Pyrethroid insecticide	0.01 - 0.5: Apples, apricots, grapes, beans, cotton seed, cruciferae, groundnuts, nuts, maize, onions, peaches, pears, peas, plums, potatoes, sorghum, tomatoes and wheat.
Cyhalothrin, lamba	Pyrethroid insecticide	0.01 - 0.5: Apples, apricots, beans, broccoli, Brussels sprouts, cabbage, cauliflower, grapes, groundnuts, maize, onions, peaches, pears, peas, plums, potatoes, sorghum, tomatoes, wheat and nuts
Cypermethrin	Pyrethroid insecticide	0.05 - 0.1: Beans, broccoli, Brussels sprouts, cabbage, cauliflower, cottonseed, grapes, groundnuts, nuts, peas and plums. 0.2 - 1: Apples, citrus, maize, peaches, pears, green Rooibos tea, tomatoes and wheat. 2: Dried rooibos tea
Cypermethrin, alpha	Pyrethroid insecticide	0.02-0.05: groundnuts, cotton seed, grapes, nuts, potatoes, sugar cane and wheat. 0.1 - 0.5: Beans, broccoli, Brussels sprout, cabbage, cauliflower, maize, peaches, pears, peas and tomatoes
Cypermethrin, beta	Pyrethroid insecticide	0.05 - 0.5: Apples, beans, citrus, cruciferae, grapes, groundnuts, nuts, maize, peaches, pears, peas, plums, sorghum, tomatoes and wheat.

Chemical	Pesticide notes	South African MRL (mg kg^{-1}) and associated crops
Cypermethrin, zeta	Pyrethroid insecticide	0.05 - 0.5: Apples, beans, broccoli, Brussels sprouts, cabbage, cauliflower, cotton seed, grapes, nuts, maize, peaches, pears, peas, sorghum, tomatoes and wheat.
Deltamethrin	Pyrethroid insecticide	0.05: Cactus pears, groundnuts, mangoes, onions, potatoes, sweet potatoes and tomatoes. 0.1 - 0.2: Apples, beans, broccoli, Brussels sprouts, cabbage, cauliflower, cotton seed, grapes, lettuce, maize, paprika, peaches, pears, plums, sorghum. 1 - 5: Hops (dry), oats, rye, stored grain and wheat.
Esfenvalerate	Pyrethroid insecticide	0.05 - 0.5: Apples, beans, cotton seed, grapes, mangoes, maize, pears, peas, potatoes, sorghum, sunflower seed, tomatoes and wheat, 15: hops (dry).
Fenvalerate	Pyrethroid insecticide	0.05 - 0.1: Grapes, mangoes, wheat, peas, potatoes, tomatoes; 0.5 -1: apples, beans, cotton seed, maize, pears, sorghum, sunflower seed; 15: hops (dry)
Permethrin	Pyrethroid insecticide	0.05 - 0.5: Apples, beans, cotton seed, grapes, groundnuts, maize, pears, peas, potatoes, sorghum, soya beans and tomatoes; 2: Cereal grains
Tau-fluvalinate	Pyrethroid insecticide	0.05 - 0.2: Apples, canola, cotton seed, peaches, pears, tomatoes and wheat
Bifenthrin	Pyrethroid insecticide	0.05 - 0.2: Apples, cottonseed, maize, pears, potatoes and tomatoes.
Acrinathrin	Pyrethroid insecticide and acaricide	0.1: Apples, pears, tomatoes and hops with MRL of 10
Fluroxypyr	Pyridinecarboxylic acid	0.1 - 0.5: Fat, meat, milk and kidney
Bupirimate	Pyrimidine fungicide	0.05 - 0.5: Apples, cucurbits, mangoes and peaches
Cyprodinil	Pyrimidine fungicide	0.05 - 0.1: Apples, barley and grapes
Fenarimol	Pyrimidine fungicide	0.2: Apples and grapes
Mepiquat chloride	Quatenary ammonium plant growth regulator	1: Cotton seed
Quinoxyfen	Quinoline fungicide	0.5 - 1: Cucurbits and grapes

Chemical	Pesticide notes	South African MRL (mg kg^{-1}) and associated crops
Azoxystrobin	Strobin fungicide	0.01 - 0.05: Brussels sprouts, cabbage, maize and potatoes. 0.2 - 1: Broccoli, cauliflower, citrus, grapes, mangoes and tomatoes.
Kresoxim-methyl	Strobin Fungicide	0.01 - 0.5: Apples, citrus, cucurbits, grapes, mangoes and pears
Pyraclostrobin	Strobin Fungicide	0.1 - 0.5: Citrus and grapes
Trifloxystrobin	Strobin fungicide	0.05 - 0.5: Apples, citrus, cucurbits, grapes, maize, pears and potatoes
Chlorsulfuron	Sulfonylurea herbicide	0.05: Oats and wheat
Iodosulfuron methyl, sodium salt	Sulfonylurea herbicide	0.05: Barley and wheat
Metsulfuron-methyl	Sulfonylurea herbicide	0.05: Barley and wheat
Nicosulfuron	Sulfonylurea herbicide	0.05: Maize
Thifensulfuron-methyl	Sulfonylurea herbicide	0.05: Barley and wheat
Triasulfuron	Sulfonylurea herbicide	0.05: Barley and wheat
Tribenuron methyl	Sulfonylurea herbicide	0.05: Barley and wheat
Piperonyl butoxide	Synergist	5 - 20: Apples, apricots, beans, broccoli, Brussels sprouts, cabbage, cauliflower, cereal grains, citrus, cotton seed, cucurbits, dried fruit, dried nuts, dried vegetables, grapes, groundnuts, guavas, lettuce, oil seeds, peaches, pears, plums, sunflower seed and tomatoes
Clofentezine	Terazine insecticide	0.05- 0.2: Apples, pears and tomatoes
Captan	Thiophtalimide	15: Apples, apricots, boysenberries, celery, grapes, guavas, olives, peaches, pears, plums, quinces, spinach, strawberries, tomatoes and youngberries.
Folpet	Thiophtalimide fungicide	0.5: Tomatoes; 15: grapes
Amitraz	Triazapentadien insecticide and acaricide	0.2 - 0.5: Apples, citrus, cotton seed, tomatoes
Simazine	Triazine herbicide	0.2: Apples, grapes, maize and pears; 10: Asparagus
Atrazine	Triazine herbicide	0.05: Maize, sorghum and sugar cane
Cyanazine	Triazine herbicide	0.05: Cottonseed, maize, sugar cane and sweet corn. 0.1 -1: Peas and rooibos
Prometryn	Triazine herbicide	0.05: Cottonseed and 0.5: carrots
Terbutryn	Triazine herbicide	0.05: Groundnuts and peas

Chemical	Pesticide notes	South African MRL (mg kg^{-1}) and associated crops
Terbuthylazine	Triazine herbicide, antimicrobial and algaecide	0.05: Maize, peas and sorghum
Cyromazine	Triazine insecticides	0.05: Potatoes, 0.5: tomatoes, 2: mushrooms and 5: green beans
Pymetrozine	Triazine insecticides	0.02 - 0.05: Cabbage and cotton seed
Hexazinone	Triazinone herbicide	1: Pineapples
Metribuzin	Triazinone herbicide	0.05: Asparagus and soya beans
Sulfentrazone	Triazolone herbicide	0: Sugar cane
Florasulam	Triazolopyrimidine herbicide	0.01: Wheat
Flumetsulam	Triazolopyrimidine herbicide	0.05: Wheat
Terbacil	Uracil herbicide	1: Peaches
Thidiazuron	Urea defoliant and plant regulator	0.5: Cotton seed
Pencycuron	Urea fungicide	0.05: Potatoes
Diuron	Urea herbicide	0.05 - 0.1: Asparagus and sugar cane
Benalaxyl	Xylylalanine fungicide	0.05: Potatoes and tomatoes. 2: Grapes
Metalaxyl	Xylylalanine fungicide	0.05 - 0.5: Avocados, broccoli, Brussels sprouts, cabbage, cauliflower, pineapples, potatoes and tomatoes. 1 - 1.5: Boysenberries, citrus, grapes and youngberries

Annex 3: Target screening pesticide analysis results.

October 2013

Index	CompoundName	Formula	Mass	Mass error	Retention time	LibraryScore	Intensity
Point E							
	Prometryne	C10H19N5S	241.1361176	6.098763354	12.94875655	32	1342.766534
Point F							
	Atrazine	C8H14ClN5	215.0937733	2.098374765	10.93878475	55	13425.87635
	Spinetoram A	C42H69NO10	747.4921482	0.236474563	16.93847564	6556	1543.987645
Point G							
	Pyriproxyfen	C20H19NO3	321.1364937	6.098736454	12.94085756	67	4238.474645
Point I							
	Dodemorph	C18H35NO	281.271865	0.236452322	15.93847447	74	908.3647543
Point K							
	Imidacloprid	C9H10ClN5O2	255.0523024	0.737465332	15.93847654	81	22348.93847
Point N							
	Azoxystrobin	C22H17N3O5	403.1168209	10.73864533	12.83746464	54	747.9585464
Point O							
	Carbendazim	C9H9N3O2	191.0694767	0.783974643	16.00374646	98	153644.5097

December 2013

Index	CompoundName	Formula	Mass	Mass error	Retention time	LibraryScore	Intensity
POINT C							
	Spiroxamine	C18H35NO2	297.2667797	5.083373663	12.09837644	94	1418.059281
	Prometryne	C10H19N5S	241.1361176	7.098476521	13.90093887	75	1539
Point E							
	Spiroxamine	C18H35NO2	297.2667797	4.09756	11.90038744	100	1652.059281
	Prometryne	C10H19N5S	241.1361176	7.09585563	13.09387465	58	1234
	Pyriproxyfen	C20H19NO3	321.1364937	7.90387464	13.45768684	98	2890.938477
Point F							
	Azoxystrobin	C22H17N3O5	403.1168209	10.9847632	13.09485666	98	13245.94858
	Pyriproxyfen	C20H19NO3	321.1364937	7.0944422	14.09847564	100	3045.784598
	Atrazine	C8H14ClN5	215.0937733	4.098757566	11.09844665	66	1468.577209
	Desmethyl-pirimicarb	C10H16N4O2	224.127326	7.0955553	13.04985756	84	1367.577913
	Sethoxydim	C17H29NO3S	327.1868159	0.864063881	16.09958576	55	1072.086193
	Spirodiclofen	C21H24Cl2O4	410.105165	0.220998576	16.09585533	86	3804.744001
Point G							
	Spiroxamine	C18H35NO2	297.2667797	6.099837442	12.09484776	97	1418.059281
Point H							
	Pyriproxyfen	C20H19NO3	321.1364937	7.094857362	13.04985577	96	4098.098747
	Spinetoram B	C43H69NO10	759.4921482	0.34875662	21.09844777	94	1358.120677
	Imidacloprid	C9H10ClN5O2	255.0523024	2.09856633	15.95857643	100	42861.78966
Point I							
	Pirimicarb	C11H18N4O2	238.142976	-0.643672891	8.094847655	100	33821.65235
	Pyriproxyfen	C20H19NO3	321.1364937	7.093847655	12.98876645	78	3908.097365
Point J							
	Buprofezin	C16H23N3OS	305.1561844	-2.94736622	8.988746465	90	29346.37179
	Spinetoram B	C43H69NO10	759.4921482	0.343547698	21.09847477	90	1234.164723
Point K							
	Imidacloprid	C9H10ClN5O2	255.0523024	2.657491049	14.99883775	98	43551.34587
	Spirodiclofen	C21H24Cl2O4	410.105165	0.321178556	16.98874664	100	7562.736354
Point M							
	Tetramethrin	Tetramethrin	Tetramethrin	6.875638846	17.93874645	55	18128.90897
	Avermectin B1a	Avermectin B1a	Avermectin B	5.988746553	16.98746532	77	5912.67648
Point N							
	Azoxystrobin	C22H17N3O5	403.1168209	11.09948765	13.09488475	80	1987.839279
	Imidacloprid	C9H10ClN5O2	255.0523024	1.285766465	13.98847766	74	23456.23458
	Atrazine	C8H14ClN5	215.0937733	5.09587433	11.09498576	45	1124.545839
Point O							
	Imidacloprid	C9H10ClN5O2	255.0523024	0.78593033	16.00088747	56	12453.13407

March 2014

Index	CompoundName	Formula	Mass	Mass error	Retention time	LibraryScore	Intensity
Point C							
	Azoxystrobin	C22H17N3O5	403.1168209	12.8529447	15.77387525	75	1676.839279
	Cypermethrin	C22H19Cl2NO3	415.0741991	0.876954522	15.57227689	98	2202.188302
	Spiroxamine	C18H35NO2	297.2667797	0.879575652	12.98775644	98	1418.059281
	Prometryne	C10H19N5S	241.1361176	0.879955333	13.09687544	98	1657.65769
	Pyriproxyfen	C20H19NO3	321.1364937	0.875664534	11.09867676	100	3209.987646
Point D							
	Azoxystrobin	C22H17N3O5	403.1168209	12.35467274	14.85963524	85	1678.987464
	Pyriproxyfen	C20H19NO3	321.1364937	0.98765432	12.09857565	100	2845.098568
Point E							
	Cypermethrin	C22H19Cl2NO3	415.0741991	0.657842342	15.9867644	86	2198.098766
	Sethoxydim	C17H29NO3S	327.1868159	0.867549563	9.09867644	54	987.9867675
	Pyriproxyfen	C20H19NO3	321.1364937	0.987656432	12.09857564	67	1224.09586
	Desmethyl-pirimicarb	C10H16N4O2	224.127326	0.87766444	13.0967744	76	142398667
Point F							
	Sethoxydim	C17H29NO3S	327.1868159	0.987564533	8.098675454	45	756.0968675
	Atrazine	C8H14ClN5	215.0937733	2.0958766	10.985766	75	13546.96877
	Imidacloprid	C9H10ClN5O2	255.0523024	0.568779888	14.8795555	100	30987.57688
Point G							
	Azoxystrobin	C22H17N3O5	403.1168209	12.86578354	13.98746452	100	2354.987465
	Cypermethrin	C22H19Cl2NO3	415.0741991	0.759863563	14.98576563	100	2987.875655
	Tetramethrin	C19H25NO4	331.1783586	9.85576321	15.90866744	33	17654.98575
Point H							
	Pirimicarb	C11H18N4O2	238.142976	-0.688856072	8.61296887	92	33821.65235
	Buprofezin	C16H23N3OS	305.1561844	-0.2387579	9.09857632	98	23655.09687
	Sethoxydim	C17H29NO3S	327.1868159	0.786964654	9.870785633	100	3456.097868
Point I							
	Spiroxamine	C18H35NO2	297.2667797	0.789875433	11.9875643	54	908.0986765
	Prometryne	C10H19N5S	241.1361176	0.785645324	13.98756534	76	1654.098756
	Pyriproxyfen	C20H19NO3	321.1364937	0.987564323	13.09857664	87	2987.985756
Point J							
	Carbendazim	C9H9N3O2	191.0694767	0.89745532	12.09874664	98	1647.049587
	Acetochlor	C14H20ClNO2	269.1182568	0.98756632	14.85957333	76	2789.049486
	Imidacloprid	C9H10ClN5O2	255.0523024	0.875653221	12.98575644	85	32987.04957
	Thiabendazole	C10H7N3S	201.0360691	0.984754532	16.9837464	100	46575.09856
	Imidacloprid-Olefin	C9H8ClN5O2	253.0366524	0.65749333	13.47646133	75	22345.09846
Point K							
	Imidacloprid	C9H10ClN5O2	255.0523024	0.756433893	13.09686756	98	38975.09877
	Pirimicarb	C11H18N4O2	238.142976	-0.688856072	8.61296887	92	33821.65235
	Buprofezin	C16H23N3OS	305.1561844	0.85765321	8.968574321	87	30987.04986
Point M							
	Desmethyl-pirimicarb	C10H16N4O2	224.127326	5.059687754	7.094855766	56	1245.059666
	Tetramethrin	C19H25NO4	331.1783586	2.098463333	14.98574633	75	18985.94857
	Avermectin B1a	C48H72O14	872.4922078	3.4050956	14.0596876	57	5647.059857
Point N							
	Imidacloprid	C9H10ClN5O2	255.0523024	0.576837365	12.90685746	54	985.9687755
	Spiroxamine	C18H35NO2	297.2667797	0.798664534	12.09875444	100	1598.098675
	Prometryne	C10H19N5S	241.1361176	0.867653534	12.09857644	98	2098.987645
Point O							
	Tetramethrin	C19H25NO4	331.1783586	0.74653321	14.98575644	100	23453.09586
	Imidacloprid	C9H10ClN5O2	255.0523024	-0.687565322	13.09847562	98	42861.13407
	Desmethyl-pirimicarb	C10H16N4O2	224.127326	4.96867765	8.059968675	75	1476.096868
	Sethoxydim	C17H29NO3S	327.1868159	0.798855674	8.09877553	85	2365.987676
	Spirodiclofen	C21H24Cl2O4	410.105165	0.675883933	16.9857563	100	4059.967662

May 2014

Index	CompoundName	Formula	Mass	Mass error	Retention time	LibraryScore	Intensity
POINT C							
	Spiroxamine	C18H35NO2	297.2667797	7.098864552	12.03499586	100	1381.099874
POINT E							
	Spiroxamine	C18H35NO2	297.2667797	8.54567383	11.746533	96	7063.099848
	Prometryne	C10H19N5S	241.1361176	7.765643524	12.9887466	66	1435.098475
POINT F							
	Azoxystrobin	C22H17N3O5	403.1168209	12.99844764	12.899484	95	142536.9986
	Atrazine	C8H14ClN5	215.0937733	2.998474522	12.00984776	75	1543.875653
	Spirodiclofen	C21H24Cl2O4	410.105165	0.3846521	24.09585877	82	4098.487566
	Spinetoram A	C42H69NO10	747.4921482	0.39875635	20.98485762	78	3785.958765
POINT G							
	Pyriproxyfen	C20H19NO3	321.1364937	6.9486352	12.98847764	93	2435.098746
POINT H							
	Spirodiclofen	C21H24Cl2O4	410.105165	0.26454578	24.95898574	95	2744.758623
POINT I							
	Dodemorph	C18H35NO	281.271865	0.4882227	14.99877565	85	124354.0969
	Pirimicarb	C11H18N4O2	238.142976	-0.688856072	8.908876453	93	2345.576253
	Pyriproxyfen	C20H19NO3	321.1364937	7.9447351	12.00949489	86	3462.958764
POINT J							
	Buprofezin	C16H23N3OS	305.1561844	-2.929023994	8.904988766	90	2847564.988
	Spinetoram B	C43H69NO10	759.4921482	0.346315345	21.99847564	94	3425.857653
POINT K							
	Imidacloprid	C9H10ClN5O2	255.0523024	0.847655411	14.99585776	100	46589.09086
	Spirodiclofen	C21H24Cl2O4	410.105165	0.356516701	23.9485762	76	7758.938456
POINT M							
	Avermectin B1a	Avermectin B1a	Avermectin B	5.77635411	16.00948732	74	36445.95876
POINT N							
	Azoxystrobin	C22H17N3O5	403.1168209	13.9847653	12.99857545	75	8756.938477
	Imidacloprid	C9H10ClN5O2	255.0523024	0.89847652	15.99585746	90	2756.948676
	Atrazine	C8H14ClN5	215.0937733	4.9958564	10.8595743	65	2647585992
POINT O							
	Carbendazim	C9H9N3O2	191.0694767	0.884762411	13.84756367	98	5876.956876

July 2014

Index	CompoundName	Formula	Mass	Mass error	Retention time	LibraryScore	Intensity
POINT C							
	Prometryne	C10H19N5S	241.1361176	6.9388474	12.998855	98	1898.364536
Point E							
	Prometryne	C10H19N5S	241.1361176	6.4874643	12.09983475	76	14352.48474
Point F							
	Azoxystrobin	C22H17N3O5	403.1168209	11.8474432	12.0394955	98	14253.98746
	Spirodiclofen	C21H24Cl2O4	410.105165	0.29844644	16.0949855	65	4010.847747
	Spinetoram A	C42H69NO10	747.4921482	0.3984743	13.498555	73	1700.398477
Point G							
	Pyriproxyfen	C20H19NO3	321.1364937	8.74645532	13.04955573	98	3345.095575
Point H							
	Spinetoram B	C43H69NO10	759.4921482	0.39484765	13.99405958	65	1400.948464
	Imidacloprid	C9H10ClN5O2	255.0523024	2.098765342	12.03949858	89	38957.09449
Point I							
	Pyriproxyfen	C20H19NO3	321.1364937	7.984745433	13.46558209	99	3900.847465
Point J							
	Buprofezin	C16H23N3OS	305.1561844	-0.5464848	8.30956432	96	25674.04947
	Spinetoram B	C43H69NO10	759.4921482	0.346315345	13.09853352	98	1456.049485
Point K							
	Imidacloprid	C9H10ClN5O2	255.0523024	3.49857632	12.39595864	87	40876.49488
	Spirodiclofen	C21H24Cl2O4	410.105165	0.237645547	16.45987351	98	82435.94949
Point M							
	Tetramethrin	Tetramethrin	Tetramethrin	7.849487652	14.95985753	65	18890.37645
	Avermectin B1a	Avermectin B1a	Avermectin B	6.100023874	16.9457353	76	7698.984464
Point N							
	Atrazine	C8H14ClN5	215.0937733	5.039844431	11.35467865	22	1098.094876

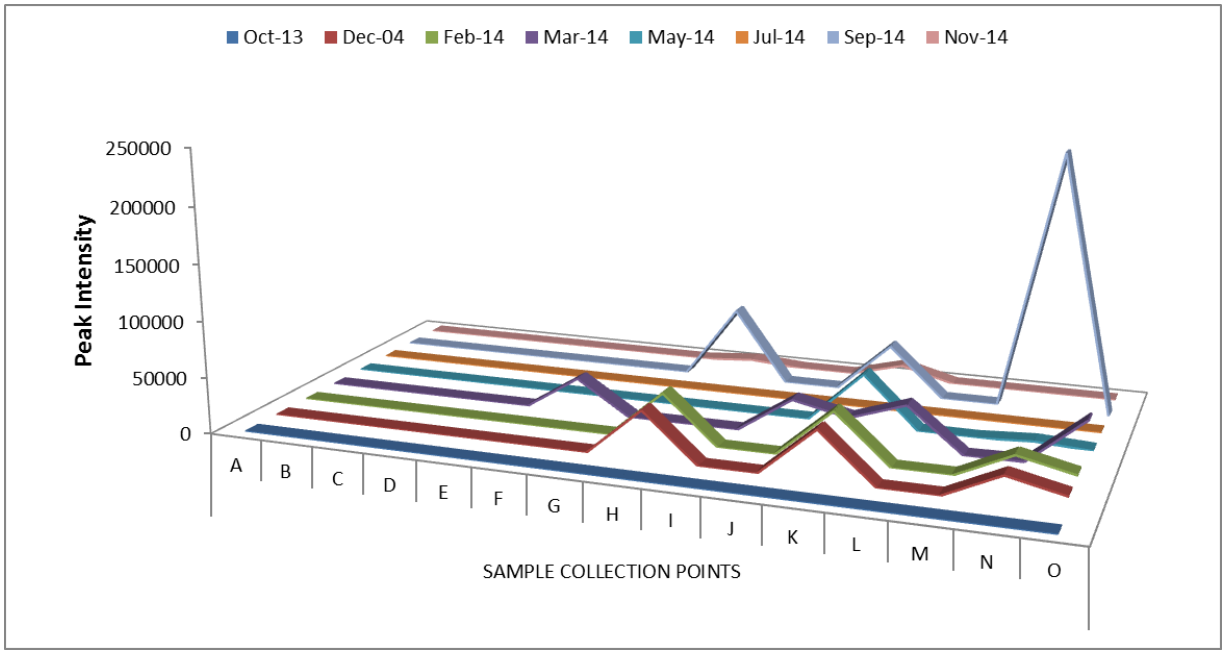
September 2014

Index	CompoundName	Formula	Mass	Mass error	Retention time	LibraryScore	Intensity
POINT C							
	Spiroxamine	C18H35NO2	297.2667797	6.90884764	11.8746453	98	14267.94956
Point E							
	Spiroxamine	C18H35NO2	297.2667797	7.859587554	12.99837464	90	1283.049585
Point F							
	Azoxystrobin	C22H17N3O5	403.1168209	12.48755	14.99847653	98	13.9485875
	Spinetoram A	C42H69NO10	747.4921482	0.294875633	17.90098457	85	16879.90949
Point G							
	Pyriproxyfen	C20H19NO3	321.1364937	6.948857632	12.03948476	98	342276.3849
Point H							
	Spinetoram B	C43H69NO10	759.4921482	0.342424568	16.97476632	92	1358.120677
	Imidacloprid	C9H10ClN5O2	255.0523024	3.746467882	15.99857446	98	65432.77465
Point I							
	Dodemorph	C18H35NO	281.271865	0.546738339	14.95857632	98	1342566.848
	Pirimicarb	C11H18N4O2	238.142976	-0.637474352	7.004985573	92	453627.8576
	Pyriproxyfen	C20H19NO3	321.1364937	7.990484875	11.99998746	67	34527.06977
Point J							
	Buprofezin	C16H23N3OS	305.1561844	-3.998565332	6.940448572	86	30376.90868
Point K							
	Imidacloprid	C9H10ClN5O2	255.0523024	0.746535388	14.99998475	87	45098.74652
	Spirodiclofen	C21H24Cl2O4	410.105165	0.32678459	13.9948476	100	6547.094487
Point M							
	Avermectin B1a	Avermectin B1a	Avermectin B	7.998484762	16.93874463	65	54637.99485
Point N							
	Imidacloprid	C9H10ClN5O2	255.0523024	2.997847465	15.98736353	83	234548.9858
	Atrazine	C8H14ClN5	215.0937733	4.287365485	11.00987235	54	23457.85564
Point O							
	Atrazine	C8H14ClN5	215.0937733	3.948573627	11.23847446	45	908.847653

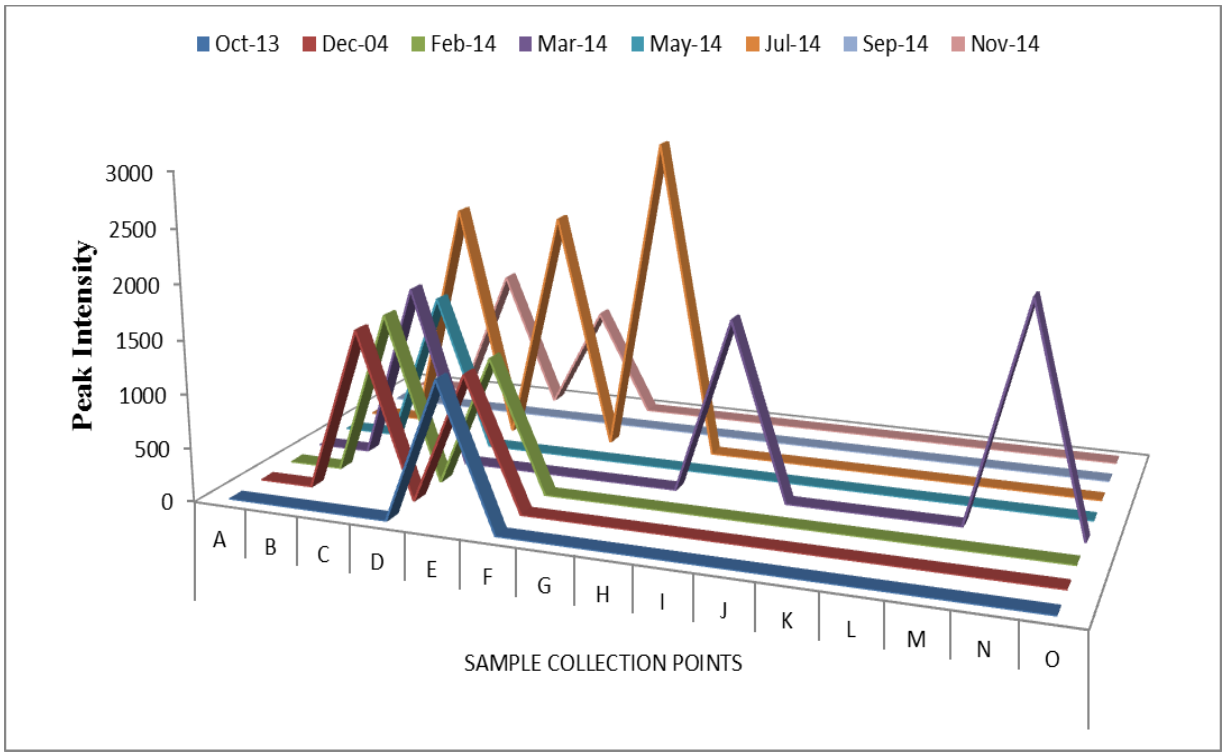
November 2014

Index	CompoundName	Formula	Mass	Mass error	Retention time	LibraryScore	Intensity
POINT C							
	Prometryne	C10H19N5S	241.1361176	0.786553533	12.84947464	76	1279.984745
Point E							
	Prometryne	C10H19N5S	241.1361176	0.687544398	11.98756535	44	981.7655844
Point G							
	Pyriproxyfen	C20H19NO3	321.1364937	5.9875643	12.94847563	65	2346.948756
Point H							
	Imidacloprid	C9H10ClN5O2	255.0523024	1.938755433	15.09876533	65	3694.746545
Point J							
	Buprofezin	C16H23N3OS	305.1561844	-1.039484756	9.983874653	56	10034.65744
	Spinetoram B	C43H69NO10	759.4921482	0.54633822	14.98765433	46	765.0393876
Point K							
	Imidacloprid	C9H10ClN5O2	255.0523024	1.998446355	12.87645357	59	12765.47658
	Spirodiclofen	C21H24Cl2O4	410.105165	0.236455789	14.87645535	68	4356.847547
Point M							
	Tetramethrin	Tetramethrin	Tetramethrin	5.876539085	16.87764536	34	12176.94884
Point N							
	Azoxystrobin	C22H17N3O5	403.1168209	9.876453222	12.09387764	49	1054.746453
	Atrazine	C8H14ClN5	215.0937733	3.098987654	13.87765443	44	765.9875653
Point O							
	Atrazine	C8H14ClN5	215.0937733	3.657483999	12.98445765	56	856.8756655
	Carbendazim	C9H9N3O2	191.0694767	0.785645338	16.34252746	72	2673.948756

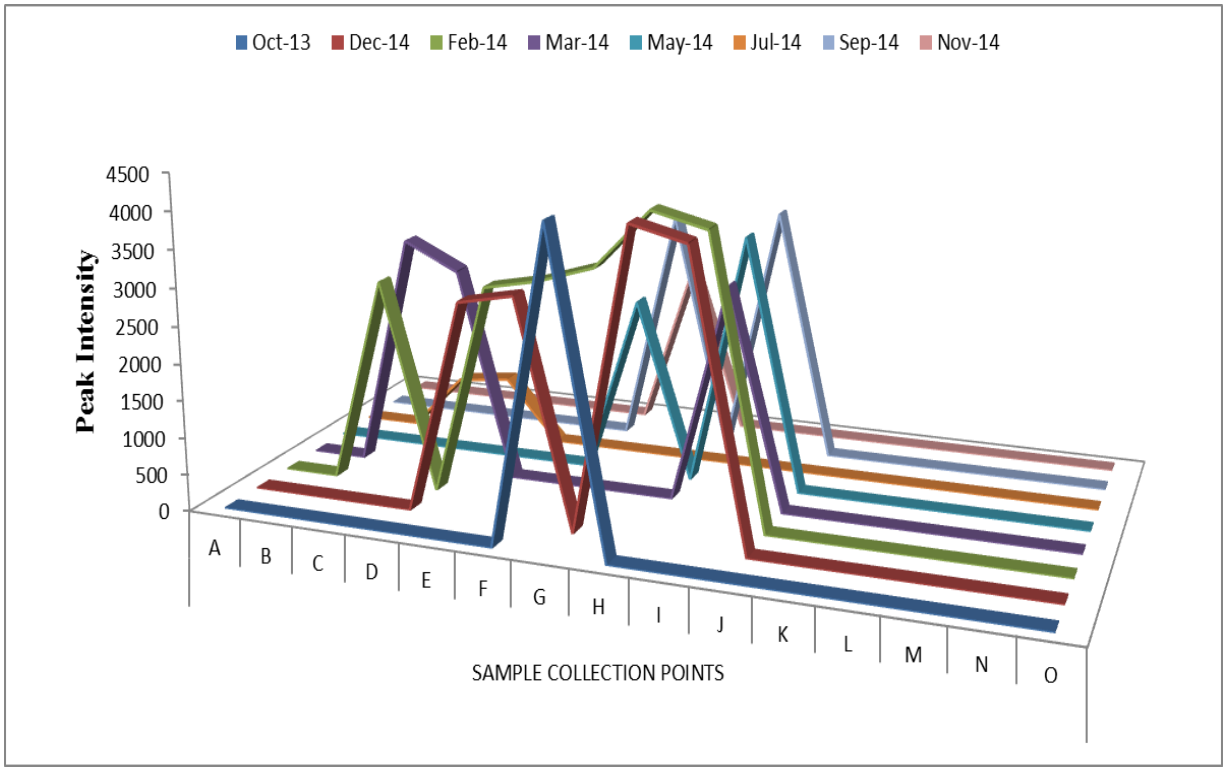
Imidacloprid Graph



Prometryn Graph



Pyriproxyfen Graph



Annex 4: Non-Target screening pesticide analysis results.

Index	CompoundName	Formula	Mass	Mass error	Retention time	LibraryScore	Intensity
Point B	Point B						
	Hexazinone	C12H20N4O2	252.1586261	0	9.756322	100	70094.94886
	Isoprocarb	C11H15NO2	193.1102789	4.164148511	7.890574611	34	1453.096857
Point C							
	DEET	C12H17NO	191.1310143	2.931235739	8.48973514	80	4781.41244
	Hexazinone	C12H20N4O2	252.1586261	0	9.756322	100	57632.90858
	Atrazine-desethyl	C6H10N5Cl	187.0624732	7.129052174	15.9857563	80	2088.512869
Point D							
	Diphenylamine	C12H11N	169.0891494	2.776578937	15.52397201	89	3284.965892
	Hexazinone	C12H20N4O2	252.1586261	-0.599479535	8.99685763	89	34567.8676
	Fenobucarb	C12H17NO2	207.125929	3.59506607	8.229715154	100	3097.683994
Point E							
	Isoprocarb	C11H15NO2	193.1102789	5.95876222	7.948475653	56	1654.485767
Point F							
	DEET	C12H17NO	191.1310143	2.103948575	9.049857463	98	4781.41244
	Isoprocarb	C11H15NO2	193.1102789	4.968757322	6.097865743	76	1875.069698
Point G							
	Cinerin I	C20H28O3	316.203845	1.986563	13.9755633	100	2345.906587
	Warfarin	C19H16O4	308.1048592	0.546756868	13.04958575	56	18765.05967
	Imazamethabenz-methyl	C16H20N2O3	288.1473927	0	13.89252472	58	1218.426458
Point H							
	Atrazine-desethyl	C6H10N5Cl	187.0624732	8.059568675	15.09585765	45	2088.512869
	Atrazine-desethyl-2-hydroxy	C6H11ON5	169.0963601	4.826243612	9.499750143	92	1621.238154
Point I							
	DEET	C12H17NO	191.1310143	0.857937763	9.094585733	100	5673.096868
	Cyromazine	C6H10N6	166.0966944	1.942442659	15.15047433	78	4748.517567
	Isoprocarb	C11H15NO2	193.1102789	2.98675764	7.96048332	85	2839.986746
Point J							
	Cinerin I	C20H28O3	316.203845	2.09585743	14.756333	85	1387.09875
	Isoprocarb	C11H15NO2	193.1102789	0.9857463	6.0978844	98	4867.098788
	Hexazinone	C12H20N4O2	252.1586261	-0.65748333	9.84746522	75	5467.75884
Point K							
	Hexazinone	C12H20N4O2	252.1586261	-0.209686774	9.564849922	98	74658.78565
	Promecarb	C12H17NO2	207.125929	0.85763333	9.857576333	100	5093.458576
	Isoprocarb	C11H15NO2	193.1102789	0.67589933	7.568474654	98	4984.069987
Point L							
	Promecarb	C12H17NO2	207.125929	2.0948755	9.0958567	87	4059.968686
	Fenobucarb	C12H17NO2	207.125929	2.98745	8.95087422	100	2938.05575
	Atrazine-desethyl	C6H10N5Cl	187.0624732	6.094857653	15.8475633	76	3985.096867

March 2014

Point B	Point B						
	Isoprocarb	C11H15NO2	193.1102789	4.164148511	7.890574611	34	1453.096857
	Hexazinone	C12H20N4O2	252.1586261	0	9.756322	100	5978.956857
Point F							
	Isoprocarb	C11H15NO2	193.1102789	5.95876222	7.948475653	56	1654.485767
	Hexazinone	C12H20N4O2	252.1586261	-0.599479535	8.99685763	89	6754
	DEET	C12H17NO	191.1310143	2.103948575	9.049857463	98	4781.41244
	Isoprocarb	C11H15NO2	193.1102789	4.968757322	6.097865743	76	1875.069698
	Hexazinone	C12H20N4O2	252.1586261	-0.209686774	9.564849922	98	4342.078
Point G							
	Cinerin I	C20H28O3	316.203845	1.986563	13.9755633	100	2345.906587
	DEET	C12H17NO	191.1310143	0.857937763	9.094585733	100	5673.096868
	Cyromazine	C6H10N6	166.0966944	1.942442659	15.15047433	78	4748.517567
	Isoprocarb	C11H15NO2	193.1102789	2.98675764	7.96048332	85	2839.986746
Point J							
	Cinerin I	C20H28O3	316.203845	2.09585743	14.756333	85	1387.09875
	Isoprocarb	C11H15NO2	193.1102789	0.9857463	6.0978844	98	4867.098788
	Hexazinone	C12H20N4O2	252.1586261	-0.65748333	9.84746522	75	5467.75884
Point K							
	Diphenylamine	C12H11N	169.0891494	0.8957554	15.94857563	100	4567.097887
	Hexazinone	C12H20N4O2	252.1586261	-0.599479535	9.227317817	89	6932.648071
	Isoprocarb	C11H15NO2	193.1102789	0.67589933	7.568474654	98	4984.069987
Point M							
	Cinerin I	C20H28O3	316.203845	3.095867362	15.00008475	86	1476.098575
	Isoprocarb	C11H15NO2	193.1102789	4.164148511	7.890574611	100	15054.38388
Point N							
	Isoprocarb	C11H15NO2	193.1102789	4.164148511	7.890574611	100	15054.38388

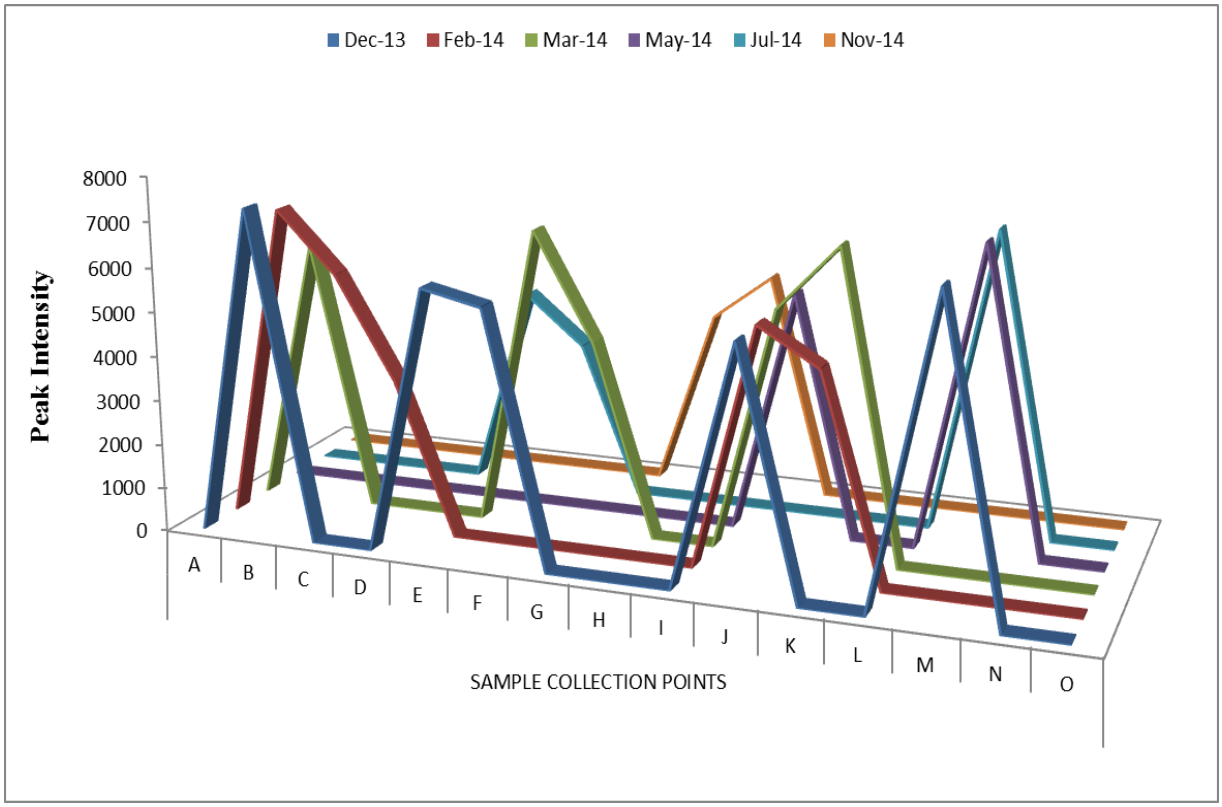
May 2013

Index	CompoundName	Formula	Mass	Mass error	Retention time	LibraryScore	Intensity
Point B							
	Isoprocarb	C11H15NO2	193.1102789	4.164148511	7.890574611	34	1453.096857
Point D							
	Diphenylamine	C12H11N	169.0891494	2.776578937	15.52397201	89	3284.965892
	Promecarb	C12H17NO2	207.125929	3.59506607	8.229715154	67	3097.683994
	Hexazinone	C12H20N4O2	252.1586261	0	9.756322	100	70094.94886
Point E							
	Isoprocarb	C11H15NO2	193.1102789	5.95876222	7.948475653	56	1654.485767
	Hexazinone	C12H20N4O2	252.1586261	-0.599479535	8.99685763	89	5978.069687
Point F							
	DEET	C12H17NO	191.1310143	2.103948575	9.049857463	98	4781.41244
	Isoprocarb	C11H15NO2	193.1102789	4.968757322	6.097865743	76	1875.069698
	Hexazinone	C12H20N4O2	252.1586261	-0.209686774	9.564849922	98	5678.07897
Point G							
	Cinerin I	C20H28O3	316.203845	1.986563	13.9755633	100	2345.906587
	Warfarin	C19H16O4	308.1048592	0.546756868	13.04958575	56	18765.05967
	Imazamethabenz-methyl	C16H20N2O3	288.1473927	0	13.89252472	58	1218.426458
	Cinerin II	C21H28O5	360.1936743	4.508265081	13.45867287	64	1006.511014
Point H							
	Atrazine-desethyl	C6H10N5Cl	187.0624732	8.059568675	15.09585765	45	2088.512869
	Cinerin I	C20H28O3	316.203845	3.095867362	15.00008475	86	1476.098575
Point I							
	DEET	C12H17NO	191.1310143	0.857937763	9.094585733	100	5673.096868
	Isoprocarb	C11H15NO2	193.1102789	2.98675764	7.96048332	85	2839.986746
Point J							
	Promecarb	C12H17NO2	207.125929	2.0948755	9.0958567	87	4059.968686
	Isoprocarb	C11H15NO2	193.1102789	0.9857463	6.0978844	98	4867.098788
	Hexazinone	C12H20N4O2	252.1586261	-0.65748333	9.84746522	75	5467.75884
Point K							
	Diphenylamine	C12H11N	169.0891494	0.8957554	15.94857563	100	4567.097887
	Promecarb	C12H17NO2	207.125929	0.85763333	9.857576333	100	5093.458576
	Isoprocarb	C11H15NO2	193.1102789	0.67589933	7.568474654	98	4984.069987
Point L							
	Fenobucarb	C12H17NO2	207.125929	2.98745	8.95087422	100	2938.05575
	Atrazine-desethyl	C6H10N5Cl	187.0624732	6.094857653	15.8475633	76	3985.096867
Point M							
	Isoprocarb	C11H15NO2	193.1102789	4.164148511	7.890574611	100	15054.38388
	Hexazinone	C12H20N4O2	252.1586261	-0.599479535	9.227317817	89	6932.648071
Point N							
	Isoprocarb	C11H15NO2	193.1102789	4.164148511	7.890574611	100	15054.38388

July 2014

Point E							
	Isoprocarb	C11H15NO2	193.1102789	5.95876222	7.948475653	56	1535.908475
	Hexazinone	C12H20N4O2	252.1586261	-0.599479535	8.99685763	89	4567.069687
Point F							
	DEET	C12H17NO	191.1310143	2.103948575	9.049857463	98	4781.41244
	Isoprocarb	C11H15NO2	193.1102789	4.968757322	6.097865743	76	1784.095858
	Hexazinone	C12H20N4O2	252.1586261	-0.209686774	9.564849922	98	3452.956857
Point M							
	Cinerin I	C20H28O3	316.203845	3.095867362	15.00008475	86	1476.098575
	Isoprocarb	C11H15NO2	193.1102789	4.164148511	7.890574611	100	1554.746523
	Hexazinone	C12H20N4O2	252.1586261	-0.599479535	9.227317817	89	4378.948764
Point N							
	Isoprocarb	C11H15NO2	193.1102789	4.164148511	7.890574611	100	1452.564733
	Atrazine-desethyl	C6H10N5Cl	187.0624732	0.987554332	14.09568675	98	4309.958675
Point O							
	Cinerin I	C20H28O3	316.203845	3.203775269	14.7736345	90	1380.142701
	Warfarin	C19H16O4	308.1048592	0.645322	13.09685764	98	3456.069687

Graph of showing Hexazinone detected at various at multiple samples sites for various collection days.



Annex 5: Fruits and Vegetables target screening pesticide analysis results

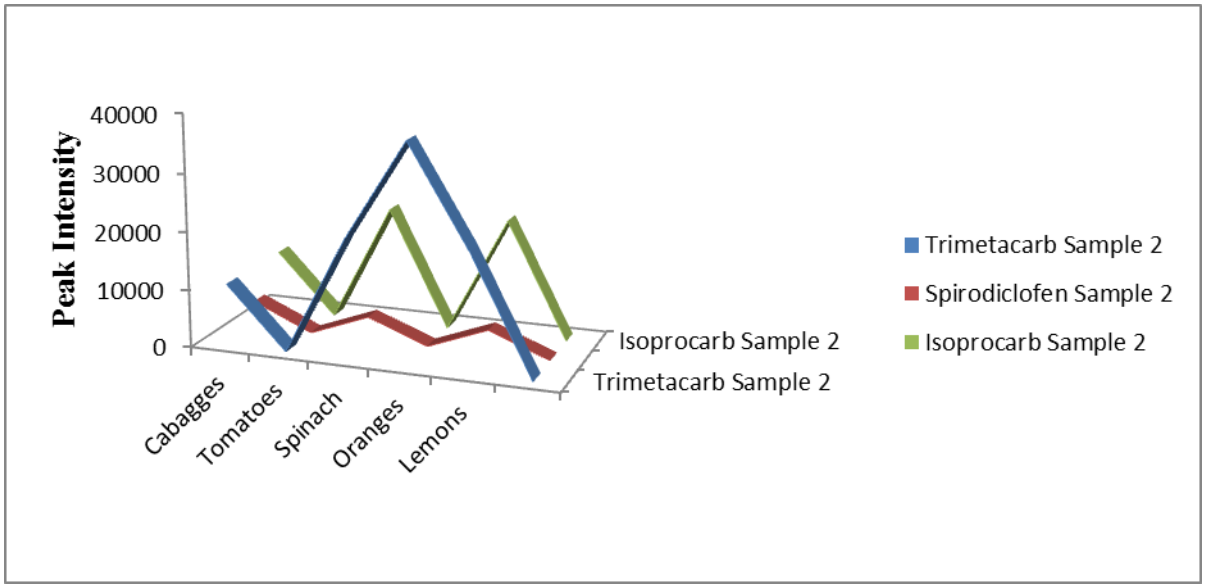
Second sample collection results

Index	CompoundName	Formula	Mass	MassError	FoundAtRT	Purity score	Intensity
TOMATOES							
	3,4,5-Trimethacarb	C11H15NO2	193.1102789	0.171836049	7.938185177	0.470508669	21874.07102
	Dimefuron	C15H19ClN4O3	338.1145684	-4.202847644	15.39527411	0.717233553	3834.191203
	Isoprocarb	C11H15NO2	193.1102789	0.171836049	7.938185177	0.470508669	21874.07102
	DEET	C12H17NO	191.1310143	-0.265318364	8.564561024	0.162345825	11096.1433
	Jasmolin I	C21H30O3	330.2194951	8.735637071	14.97089539	0.527343271	2708.014469
CABBAGE							
	Cinerin II	C21H28O5	360.1936743	-3.06284793	13.19328057	0.91818575	7043.067133
	3,4,5-Trimethacarb	C11H15NO2	193.1102789	1.458159982	7.929014014	0.449592064	10736.79269
	Isoprocarb	C11H15NO2	193.1102789	1.458159982	7.929014014	0.449592064	10736.79269
	DEET	C12H17NO	191.1310143	0.570970981	5.036865792	0.196531024	3705.097259
LEMON							
	3,4,5-Trimethacarb	C11H15NO2	193.1102789	-1.409711379	7.933852931	0.478370479	20219.3133
	Isoprocarb	C11H15NO2	193.1102789	-1.409711379	7.933852931	0.478370479	20219.3133
	DEET	C12H17NO	191.1310143	-1.172499947	8.552899418	0.110696541	13049.60721
ORANGES							
	3,4,5-Trimethacarb	C11H15NO2	193.1102789	-1.262614624	7.921745689	0.465994632	20582.43818
	Dimefuron	C15H19ClN4O3	338.1145684	-5.152578809	15.39597006	0.691080832	4712.326176
	Isoprocarb	C11H15NO2	193.1102789	-1.262614624	7.921745689	0.465994632	20582.43818
	DEET	C12H17NO	191.1310143	-1.409424056	8.535763237	0.125383338	12332.33859
SPINACH							
	Benomyl	C14H18N4O3	290.1378907	-1.355573794	9.36497416	0.812805384	10727.36198
	3,4,5-Trimethacarb	C11H15NO2	193.1102789	-1.661289319	7.923773134	0.44988199	20382.6008
	Isoprocarb	C11H15NO2	193.1102789	-1.661289319	7.923773134	0.44988199	20382.6008
	DEET	C12H17NO	191.1310143	-1.587248419	8.535262354	0.121488983	10959.78017

Third sample collection

Index	CompoundName	Formula	BaseMass	MassError	FoundAtRT	Purity score	Intensity
Tomatoes							
	Spirodiclofen	C21H24Cl2O4	410.105165	0.356516701	25.60876484	86	3804.744001
	Imidacloprid	C9H10ClN5O2	255.0523024	0.621933695	16.60684531	56	12453.13407
	Isoprocarb	C11H15NO2	193.1102789	0.171836049	7.938185177	0.470508669	21874.07102
	DEET	C12H17NO	191.1310143	-0.265318364	8.564561024	0.162345825	11096.1433
Cabagges							
	Isoprocarb	C11H15NO2	193.1102789	1.458159982	7.929014014	0.449592064	10736.79269
	Spirodiclofen	C21H24Cl2O4	410.105165	0.356516701	25.60876484	86	3804.744001
	Prometryne	C10H19N5S	241.1361176	7.765643524	12.9887466	66	1435.098475
Lemon							
	Spirodiclofen	C21H24Cl2O4	410.105165	0.356516701	25.60876484	100	4087.293485
	Pyriproxyfen	C20H19NO3	321.1364937	7.220662214	13.67898297	100	4098.098747
	Azoxystrobin	C22H17N3O5	403.1168209	12.22837562	13.74746586	89	1987.839279
Oranges							
	Imidacloprid	C9H10ClN5O2	255.0523024	1.267890365	16.13452679	76	23456.23458
	Spirodiclofen	C21H24Cl2O4	410.105165	0.356516701	25.60876484	100	4087.293485
	Spiroxamine	C18H35NO2	297.2667797	8.54567383	11.746533	96	7063.099848
Spinach							
	Prometryne	C10H19N5S	241.1361176	7.765643524	12.9887466	66	1435.098475
	Azoxystrobin	C22H17N3O5	403.1168209	13.9847653	12.99857545	75	8756.938477
	Imidacloprid	C9H10ClN5O2	255.0523024	0.89847652	15.99585746	90	2756.948676
	Atrazine	C8H14ClN5	215.0937733	4.9958564	10.8595743	65	2647585992
	Spirodiclofen	C21H24Cl2O4	410.105165	0.220998576	16.09585533	86	3804.744001

Graph showing the detected pesticides in various fruits and vegetables from second sample collection



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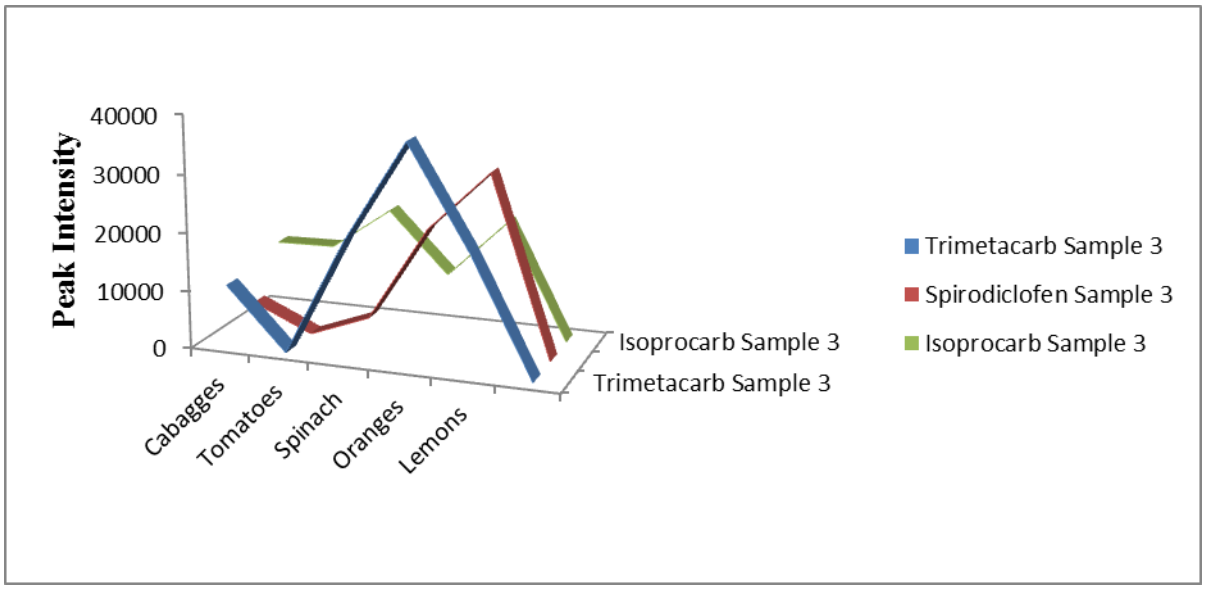
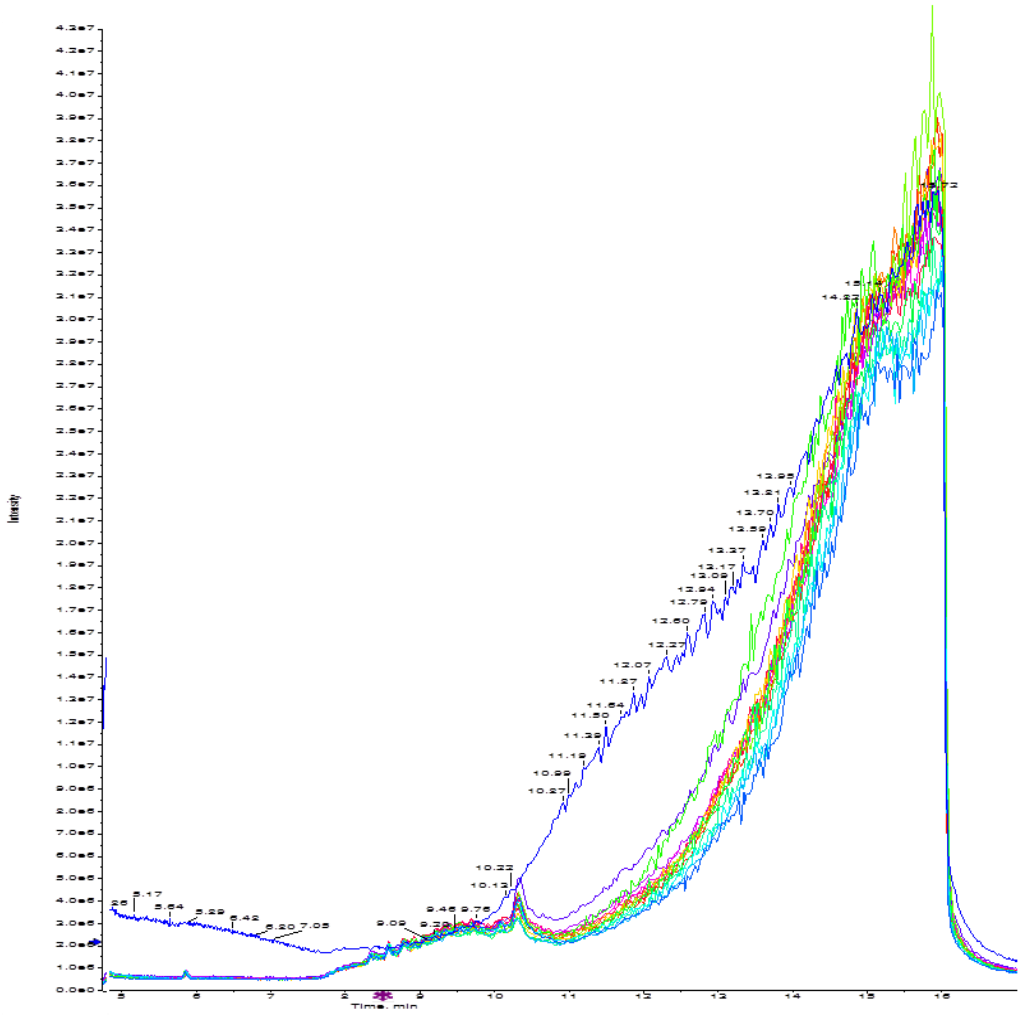
Graph showing the detected pesticides in various fruits and vegetables from third sample collection

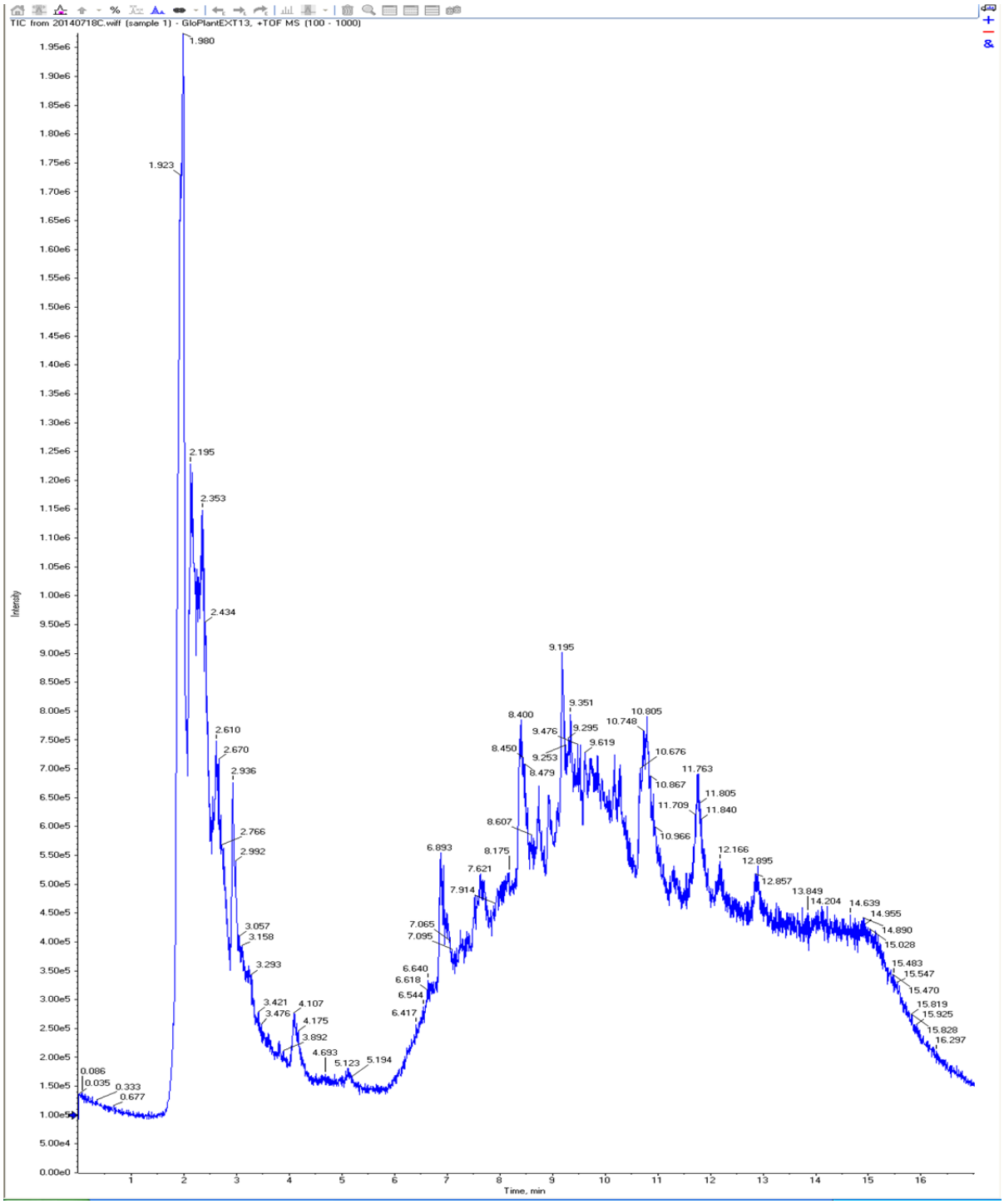


Annex 6: Pharmaceutical and Personal care products analysis results.

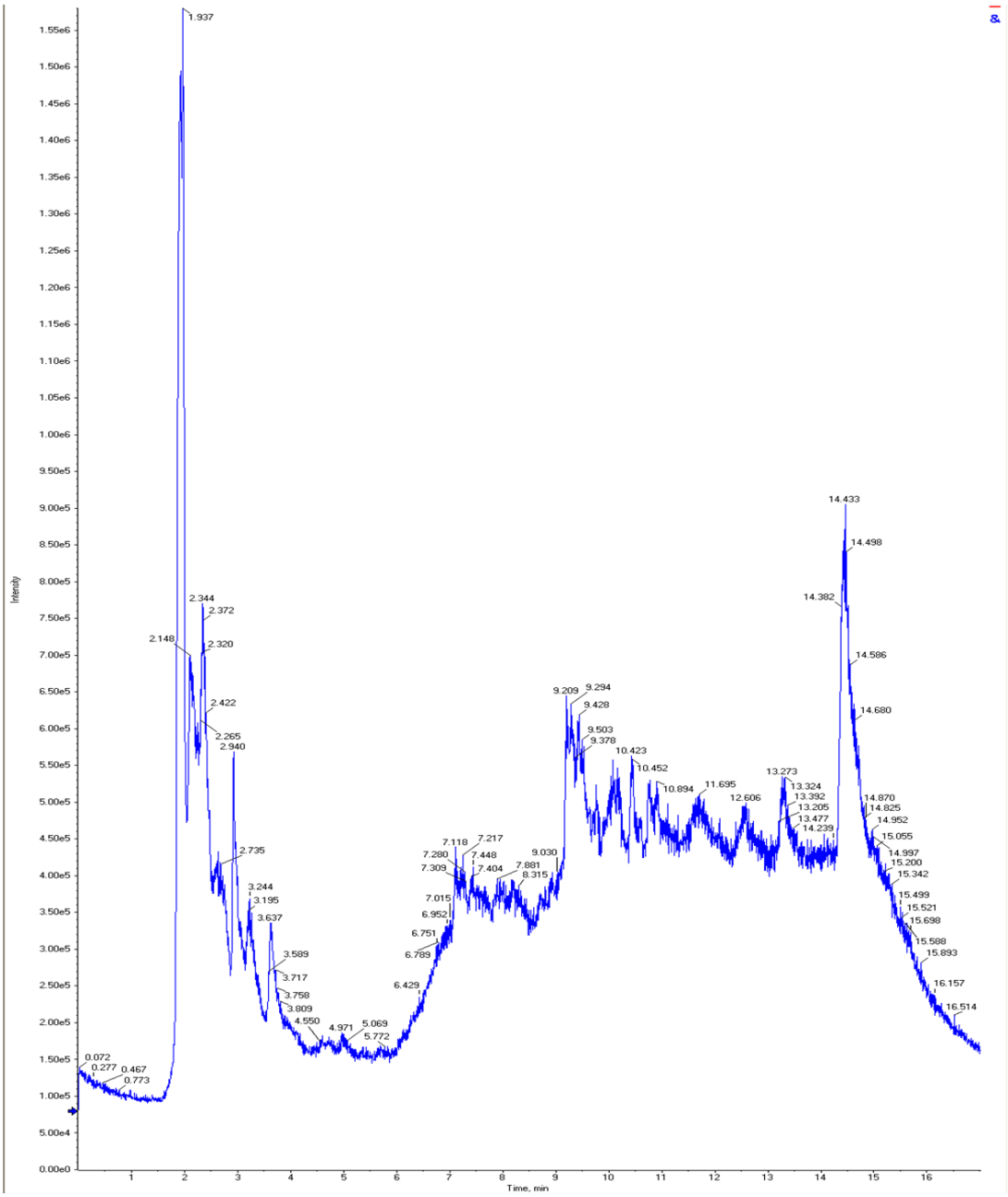
Total Ion Chromatography (TIC) obtained by combining all mass spectra from 8 samples collected during the same day, from various points along the Kat River.



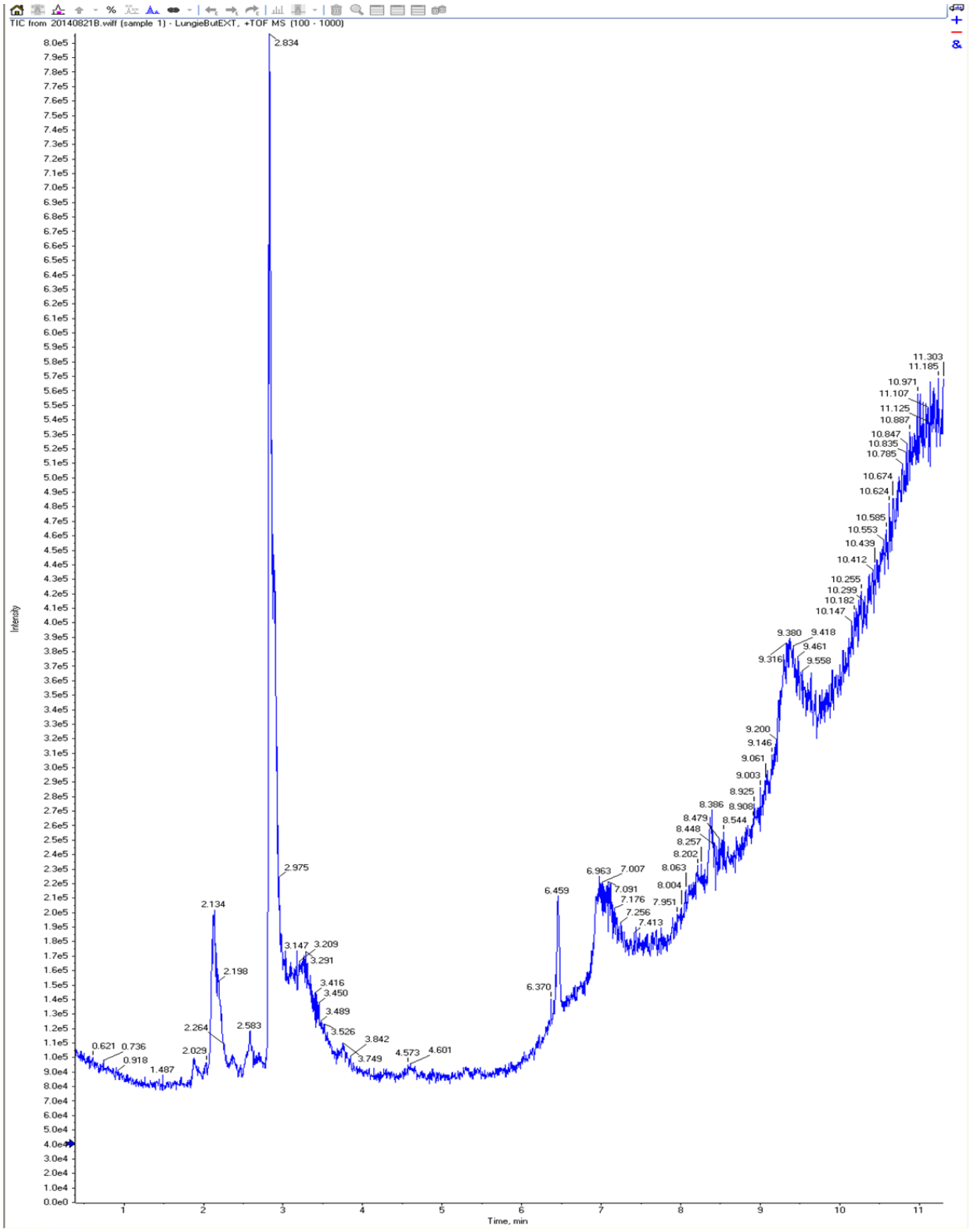
TIC of sample collected downstream Seymour waste water treatment plant



TIC of a sample collected downstream Fort Beaufort waste water treatment plant.



TIC of a sample collected after Balfour community



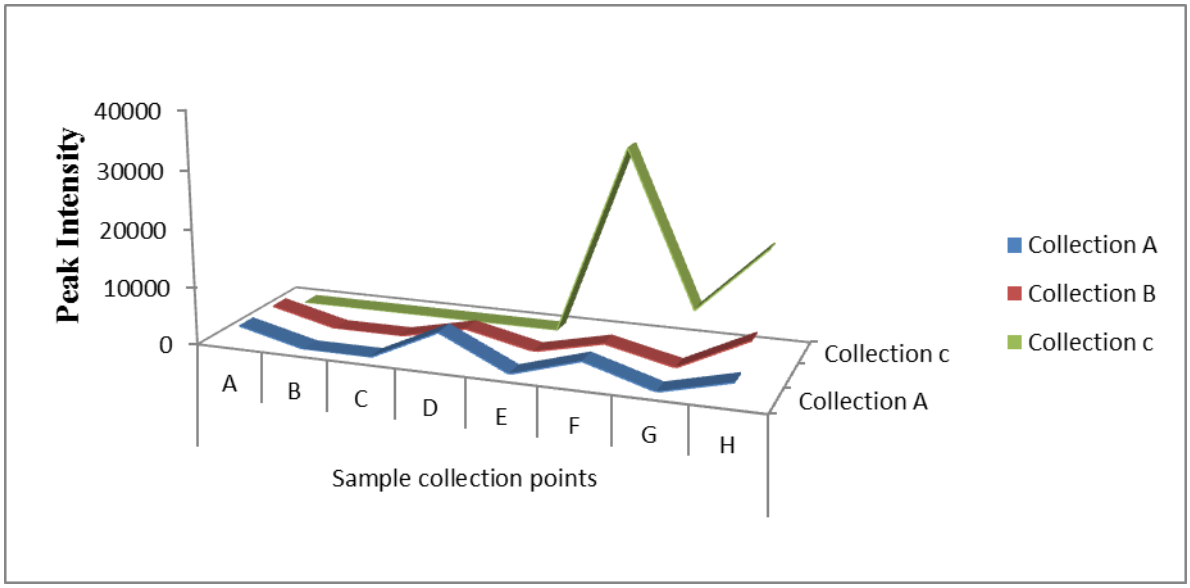
Second sample collection

Index	CompoundName	Formula	BaseMass	MassError	FoundAtRT	LibraryScore	LibraryHitName	Intensity
SAMPLE B								
	Dixyrazine	C24H33N3O2S	427.2293494	-7.822556457	9.667294087	0.455100275	Dixyrazine	4053.153813
	Paracetamol	C8H9NO2	151.0633286	0.09996	13.9874494	0.89657	Paracetamol	4287.059684
	Biotin	C10H16N2O3S	244.0881644	245.0998074	17.81575188	9.122281143	Biotin	60786.98576
	Timolol	C13H24N4O3S	316.1569127	-5.769483037	13.92992174	0.608710863	Bupirimate	2803.170907
	Pindolol	C14H20N2O2	248.1524781	-5.586016819	9.212584955	0.604766698	Pindolol	5691.182578
SAMPLE D								
	Nomifensine	C16H18N2	238.1469987	-16.51742631	8.596131133	0.217347432	Nomifensine	26693.24881
	Diaveridine	C13H16N4O2	260.127326	-5.416989933	8.607999555	1	Diaveridine	293241.4512
	Biotin	C10H16N2O3S	244.0881644	245.0998074	17.81575188	9.122281143	Biotin	4786.064352
	Paracetamol	C8H9NO2	151.0633286	0.09996	13.9874494	0.89657	Paracetamol	3456.9678455
	Gabapentin	C9H17NO2	171.125929	4.728845915	10.38843874	0.140005132	Gabapentin	1341.014116
	Mafenide	C7H10N2O2S	186.0462995	24.06888484	8.034755402	0.170333275	Carbimazole	4490.725141
SAMPLE H								
	Coniine	C8H17N	127.1360997	4.223670924	8.585081221	0.420443935	Coniine	3077.071431
	Paracetamol	C8H9NO2	151.0633286	0.01	13.9874494	0.9	Paracetamol	3977.94854
	Buprofezin	C16H23N3OS	305.1561844	-2.376983826	8.786965649	0.447476776	Buprofezin	35106.75965
	Biotin	C10H16N2O3S	244.0881644	245.0998074	17.81575188	9.122281143	Biotin	3987.5697
	Metamfepramone	C11H15NO	177.1153642	2.734446148	8.206320913	0.162300608	Metamfepramone	8179.829194
	Carbimazole	C7H10N2O2S	186.0462995	24.06888484	8.034755402	0.170333275	Carbimazole	4490.725141
	3,4-Methylenedioxymethamphetamine	C11H15NO2	193.1102789	1.913361173	7.883729155	0.456168381	alpha-Ethyl-1.3-benzodioxole-5-ethanamine	16220.08125
SAMPLE E								
	Primidone	C12H14N2O2	218.1055279	-7.590997888	8.368627492	0.20956766	Primidone	4893.024409
	N,N-Diethyl-m-toluamide	C12H17NO	191.1310143	4.26628933	8.474701563	0.240790702	phendimetrazine	7663.363712
	Hydroxymethylpyridine	C6H7NO	109.0527639	3.848369273	5.840794696	0.773264413	Hydroxymethylpyridine	1013.482763
	Metamfepramone	C11H15NO	177.1153642	2.734446148	8.206320913	0.162300608	Metamfepramone	8179.829194
SAMPLE J								
	Sulfaquinoxaline	C14H12N4O2S	300.0680976	5.39553541	13.69293976	0.928754734	Sulfaquinoxaline	2447.994456
	Cyamemazine	C19H21N3S	323.1456197	-1.440129214	15.12853796	0.808019442	Cyamemazine	85691.60239
	Pindolol	C14H20N2O2	248.1524781	-5.586016819	9.212584955	0.604766698	Pindolol	5691.182578
	Biotin	C10H16N2O3S	244.0881644	245.0998074	17.81575188	9.122281143	Biotin	9987.472625
	Metamitron	C10H10N4O	202.0854611	-13.85631001	7.471386308	0.797294894	Metamitron	2672.320391
	Oxitropium	C19H26NO4	332.1861836	-9.737333638	9.72712898	0.828013195	Oxitropium	8217.306602
	Paracetamol	C8H9NO2	151.0633286	0.0100045	13.9874494	0.8887645	Paracetamol	4045.875644

Third sample collection

Index	CompoundName	Formula	BaseMass	MassError	FoundAtRT	LibraryScore	Intensity
SAMPLE B							
	Dixyrazine	C24H33N3O2S	427.2293494	-7.214731196	9.674836898	0.522174937	3511.972777
	Paracetamol	C8H9NO2	151.0633286	0.0100045	13.9874494	0.8887645	5768.098756
	Irbesartan	C25H28N6O	428.2324598	-0.687919801	16.04240045	0.683854593	2880.307565
	Timolol	C13H24N4O3S	316.1569127	-3.348135085	13.90910948	0.552692832	5625.099091
	Biotin	C10H16N2O3S	244.0881644	245.0998074	17.81575188	9.122281143	52672.89054
	Pindolol	C14H20N2O2	248.1524781	-3.506577709	9.208119368	0.604766698	4542.999102
SAMPLE D							
	Nomifensine	C16H18N2	238.1469987	-17.63574169	8.608561252	0.206492025	33008.48382
	Trimethoprim	C13H16N4O2	260.127326	-6.303447901	8.629427032	1	336712.0839
	Paracetamol	C8H9NO2	151.0633286	0.0100045	13.9874494	0.8887645	3856.956353
	Metamfepramone	C11H15NO	177.1153642	3.777819394	8.21249553	0.152304522	8478.806627
	Pindolol	C14H20N2O2	248.1524781	-3.506577709	9.208119368	0.604766698	4542.999102
	Coniine	C8H17N	127.1360997	2.721406888	8.579076866	0.430321964	2705.283178
SAMPLE F							
	Betaine	C5H11NO2	117.0789787	2.290732109	3.939548322	0.846575673	1598.042239
	Dihydralazine	C8H10N6	190.0966944	15.17530969	15.09546709	0.130177928	6360.422298
	Paracetamol	C8H9NO2	151.0633286	0.0100045	13.9874494	0.8887645	3934.125474
	Perazine	C20H25N3S	339.1769198	-8.95212118	9.50746512	0.237677299	2574.476084
	Tocainide	C11H16N2O	192.1262633	-7.622345509	11.97264797	0.714900965	1835.784055
	Pindolol	C14H20N2O2	248.1524781	-3.506577709	9.208119368	0.604766698	4542.999102
	Oxprenolol	C15H23NO3	265.1677938	-4.286959478	12.16985552	0.679496797	1236.292181
	Carbimazole	C7H10N2O2S	186.0462995	25.43315919	8.032960396	0.170333275	5912.705058
SAMPLE G							
	3,4-Methylenedioxymethamphetamine	C11H15NO2	193.1102789	4.191347237	7.890232458	0.455789071	17785.50013
	Primidone	C12H14N2O2	218.1055279	-9.568949062	8.380806586	0.20956766	6141.887037
	Tetroxoprim	C16H22N4O4	334.1641055	-5.269123898	8.974579919	0.854426811	2019.797998
SAMPLE H							
	Paracetamol	C8H9NO2	151.0633286	0.0100045	13.9874494	0.8887645	9758.655434
	Sulfaquinoxaline	C14H12N4O2S	300.0680976	2.711692731	13.67508584	0.928754734	2540.732602
	9-Hydroxyrisperidone	C23H27FN4O3	426.2067193	-6.025010479	14.84094332	0.872181828	6269.433633
	Pindolol	C14H20N2O2	248.1524781	-3.506577709	9.208119368	0.604766698	4542.999102
	Metamitron	C10H10N4O	202.0854611	-14.38245657	7.487154868	0.749338898	4553.592005
	Oxitropium	C19H26NO4	332.1861836	-8.828646796	9.818176517	0.828013195	7869.787297

Graph showing the intensity of Biotin at various sample collection points from three different sample collections



Graph showing the intensity of Timolol at various sample collection points from three different sample collections.