DEVELOPMENT OF NOVEL FLUORESCENT SENSORS FOR THE SCREENING OF EMERGING CHEMICAL POLLUTANTS IN WATER

Report to the

Water Research Commission

by

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EXECUTIVE SUMMARY

Background

Emerging chemical pollutants (ECPs) are defined as new chemicals that do not have a regulatory status, but may have an adverse effect on human health and the environment. Sources and environmental pathways of these ECPs have been increasingly associated with waste and waste waters arising from industrial, agricultural and municipal activities. The ECPs of current concern include a wide range of compounds that include polychlorinated biphenyls, polycyclic aromatic hydrocarbons (PAHs), brominated flame retardants, antivirals, antiretrovirals, pesticides, phthalates, disinfectants, psycho-stimulants, diuretics, cosmetics, contrast media, neuroactive compounds, blood lipid lowering agents, beta blockers, antibiotics, analgesics and anti-inflammatory drugs.

There is currently a lack of information regarding the nature, concentration, variability, transport and fate of these compounds in water, which is of global concern. In order to understand the potential biological impact of these chemicals, reliable and practical methods to identify and quantify ECPs in water are urgently needed. Analysis of ECPs is complex and challenging due to the wide range of compound classes involved for which there are no established analytical methods in some cases. The sample matrix itself is often complex and may interfere with the target analyses; also, the ECPs are usually present at trace levels in water ($ng \cdot L^{-1}$ or $\mu g \cdot L^{-1}$). The analysis of water for ECPs is, however, of vital importance, as many of these compounds are known to have adverse human health effects such as endocrine disruption, chronic toxicity, and the development of pathogen resistance to medication, which make a holistic understanding of exposure and risk necessary.

Data relating to the occurrence of ECPs in South African waters is currently very limited. This can be ascribed in part to the high cost of performing the complex analyses involved. This project aimed to address this shortcoming by developing novel quantum dot (QD)-based fluorescence sensors for target ECPs of relevance in South Africa. For this proof of concept project, ECPs covering a range of compound classes were selected as target analytes. Specifically, compounds known to have environmental and human health concerns and/or are known to be used or produced in large quantities and are ubiquitous in the environment were selected, namely, PAHs (as a compound class), atrazine (a pesticide), acetaminophen (a pharmaceutical), and triclosan (a personal care product).

QDs are semiconductor crystalline nanomaterials that have unique electronic and optical properties due to quantum confinement effects. QDs have several optical properties that make them ideal fluorescence sensors. These properties include high quantum yields, high molar extinction coefficients, broad absorption with narrow and symmetric photoluminescence spectra spanning the ultraviolet visible to the near-infrared, large effective Stokes shifts and high resistance to photobleaching (high photostability). In addition, the fluorescent emission of QDs is size-tunable and they have broad excitation spectra, which allow for excitation at a single wavelength far removed from their emission.

Aims of the project

The aims of this project were thus to:

1) Prioritise ECPs in water in order to define target analytes for fluorescence screening and to identify potential water sampling sites in Gauteng and surrounds.
2) Synthesise and characterise suitable novel QDs and QD nanocomposites for use in the proposed fluorescence sensors.
3) Immobilise the QDs and QD nanocomposites in suitable polymers (such as siloxanes) in order to generate fluorescence sensor prototypes.
4) Test and optimise the fluorescence sensor materials and prototypes using synthetic standard solutions of the target compound(s) in water.

Prioritisation

In this study, a prioritisation methodology for ECPs in water was developed and used. A group of 168 ECPs were prioritised based on various factors including toxicity (acute toxicity in rats), environmental persistence (half-life in water), relevant physicochemical data (partition coefficient) and global prevalence. The ECPs were then ranked by assigning weightings to these factors. The rankings each substance obtained were then summed across all the factors to obtain a final weighting for each substance. Each substance was then ranked in accordance with its final summed total, which resulted in a ranked list of priority ECPs. After the prioritisation process, it became apparent that pesticides were a class of compounds that warranted further studies as they accounted for many of the highest ranked ECPs. The full list of ranked ECPs generated can prove to be an invaluable starting point for further research into ECPs in South African water bodies and to compare various ECPs with each other in term of their persistence, bioaccumulation and toxicity and thus the hazard they pose to the environment.

Additionally, relevant surface water sampling sites in the Gauteng Province of South Africa were identified using a geographic information system to generate maps. The sites were identified by determining potential sources of ECPs, including hospitals and clinics, sewage treatment plants, and areas with high population densities or areas vulnerable from an environmental point of view. Buffers were drawn around these areas to identify the water sources that have the highest probability of containing the relevant ECPs. The wards that are located along the identified at-risk river areas were also identified to aid decision- and policymakers within both the private sector and government in making informed decisions regarding ECPs.

Synthesis and characterisation of QDs

Two types of QDs, namely, semiconductor CdSe/ZnS QDs and graphene QDs (GQDs) were investigated to evaluate the relative merits and limitations of each in the fluorescence sensing of the target ECPs in water. Water-soluble and highly fluorescent CdSe/ZnS QDs with different thiol ligand groups as capping agents were successfully synthesised via a one-pot approach under high temperature. Characterisation by fluorescence spectroscopy, ultraviolet visible spectroscopy, powder X-ray diffraction and Fourier-transform infrared spectroscopy confirmed the formation of CdSe/ZnS QDs that were capped with either L-cysteine (L-cys), glutathione (GSH) or N-acetyl-L-cys. The synthesised QDs exhibited narrow and intense photoluminescence emission thanks to the thiol ligands as stabilisers and also due to the formation of a ZnS shell on the CdSe core that has a higher band gap than CdSe. The shell also reduces the possibility of toxic Cd leaching from the QD core during use.

GQDs were successfully prepared and tested for application as unique fluorescence sensors for PAHs. Compared with semiconductor QDs, GQDs have the advantages of low toxicity and strong affinity to PAHs because of π-π interactions. The GQDs were prepared by the oxidation of graphite flakes as precursors to form graphene oxide, which was further oxidised to form the GQDs. The optical properties of these GQDs were investigated and they were found to have strong fluorescence for which the intensity and emission wavelength are dependent on the excitation wavelength. The probe was then tested for sensing selected PAHs, namely, phenanthrene, anthracene, pyrene and naphthalene. These PAHs are commonly found in water systems due to their higher water solubility than other PAHs. They are ubiquitous in the environment and have been listed among priority PAHs by the United States Environmental Protection Agency (US EPA).

Immobilisation of QDS

A preliminary investigation into the immobilisation of QD sensors in a silicone-based polymer was undertaken, where polydimethylsiloxane was used to immobilise hydrophobic QDs and thin film materials were fabricated on glass slides via spin-coating. These materials were fluorescent; however, their fluorescence intensity was poor. Therefore, ways to improve the immobilisation method need to be explored.

Testing and optimising the fluorescence sensor materials and prototypes using synthetic standard solutions of the target compound(s) in water

Aqueous L-cys and GSH-capped CdSe/ZnS QDs were applied as fluorescence sensors for acetaminophen and triclosan respectively. A simple and rapid method was developed based on the fluorescence enhancement of aqueous CdSe/ZnS QDs in the presence of acetaminophen and triclosan. Under optimal conditions using L-cys-capped CdSe/ZnS QDs, ΔF ($F-F_0$) was linearly proportional to acetaminophen concentration from 3.0×10^{-9}–1.0×10^{-7} mol·L^{-1} with a limit of detection (LOD) of 6.0 nmol·L^{-1}, whilst the dynamic range and LOD utilising aqueous CdSe/ZnS-GSH QDs for triclosan sensing were found to be 1.0×10^{-8}–5×10^{-8} mol·L^{-1} and 15.0 nmol·L^{-1} respectively for the plot of F/F_0 versus triclosan concentration.

Pesticide sensing experiments were conducted using different QDs as fluorescence probes with atrazine as the target pesticide. Interaction of the probes with atrazine resulted in enhancement of the QD fluorescence signal, suggesting that a ‘turn-on’ probe can be developed. CdTeSe/ZnSe/ZnS QDs that gave initial LODs of 1.7×10^{-7} mol·L^{-1} were chosen as suitable probes for further optimisation because of their intense fluorescence signal and reduced toxicity.

Sensing studies for selected PAHs were carried out using GQDs as sensor material. Interacting GQDs with PAHs resulted in quenching of the fluorescence signal as the PAH concentration increased. Promising results were obtained with phenanthrene with the probe providing an LOD of 2.5×10^{-8} mol·L^{-1}. In addition, a synthetic route to couple L-cys-CdSeTe/ZnSe/ZnS QDs to graphene to form a stable novel nanocomposite was successfully completed to form a suitable sensor for PAHs in water. An LOD of 0.19 µg·L^{-1} was obtained for phenanthrene under optimum conditions, while the LOD of anthracene, pyrene and naphthalene were estimated to be approximately 0.26 µg·L^{-1}.

Finally, a cost benefit analysis was conducted for using QD fluorescence sensors. An evaluation of the costs incurred towards synthesising the probes was compared with costs of current conventional methods, such as field gas chromatography and immunoassay test kits, which showed that QD sensors may provide a cost-effective alternative.

Concluding comments

Based on the characterisation of the nanomaterials synthesised and tested using solutions of ECP standards, promising results were obtained regarding the novel fluorescence sensors developed in this proof of concept study. It should be noted that regardless which definition of ECPs is considered, the compounds selected as target analytes for fluorescence sensor development in this research are certainly of environmental concern in the South African as well as in the global context. Thus, the development of our novel analytical methods may prove to be a significant contribution towards enabling more widespread monitoring of these pollutants. We have shown that the novel fluorescence sensors we developed have the potential to provide complimentary information to standard analytical approaches and can thus enable wider monitoring of target ECP contamination in South African water in a cost-effective manner. The sensors may also find application in determining the effectiveness of waste water treatment processes in the removal of these ECPs. It is thus envisaged that our sensors may assist in future in enhancing the sound management of our water resources.

Recommendations for future research

It is necessary that the technologies developed in this initial project be applied to the monitoring of the target ECPs in real water samples to optimise and validate the results and to determine effects of various variables and parameters (such as pH; contact time and interferences) on their performance. Enhanced selectivity via surface modification of the QDs should be investigated. In addition, further work is needed to optimise the immobilisation of the nanomaterials to enable reuse.

Capacity building and knowledge dissemination

One BSc Hons (Chemistry) and one MSc (Water Resource Management) student graduated based on research projects directly related to this project. In addition, two students are currently working towards their PhD (Chemistry) degrees based on research presented in this report. Four journal articles arising from this work have been published to date; another has been accepted for publication. Two oral conference presentations were given on this work: one at a local conference by a student and the other was a plenary talk by the project leader at an international conference.

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2,4-DCP	2,4-Dichlorophenol
2,4,6-TCP	2,4,6-Trichlorophenol
β-CD	β-cyclodextrin
β-CD/GNP	β-Cyclodextrin Graphene Nano Platelet
AA	Ascorbic Acid
AChE	Acetylcholinesterase
AP	4-Aminophenol
API	Active Pharmaceutical Ingredient
APTES	3-Aminopropyltriethoxysilane
ARV	Anti-retroviral
AuNP	Gold Nanoparticle
BFR	Brominated Flame Retardant
CA	Calixarene
CAS	Chemical Abstract Service
CDs	Cyclodextrins
ChOx	Choline Oxidase
CNS	Central Nervous System
CNT	Carbon Nanotube
CTAB	Cetyltrimethylammonium Bromide
CV	Cyclic Voltammetry
Cys	Cysteamine
DA	Dopamine Hydrochloride
DBP	Disinfectant By-product
DDE	Dichloro-Diphenyl-Dichloroethylene
DDT	Dichloro-Diphenyl-Trichloroethane
DEET	N,N-Diethyl-Meta-Toluamide
Dic	Diclofenac Sodium Salt
DNA	Deoxyribonucleic Acid
ECP	Emerging Chemical Pollutant
EDC	Endocrine-disrupting Chemical
EE2	Ethinylestradiol
EP	Epinephrine Hydrochloride
EPA	Environmental Protection Agency
ES	Oestradiol
ESI	Electrospray Ionization
ESRI	Environmental Systems Research Institute
FRET	Förster Resonance Energy Transfer
FT-IR	Fourier-transform Infrared Spectroscopy
FWHM	Full Width at Half Maximum
GABA	Gamma-aminobutyric Acid
GCE	Glassy Carbon Electrode
GC–MS	Gas Chromatography–Mass Spectrometry
GC–MS/MS	Gas Chromatography–Tandem Mass Spectrometry
GIS	Geographic Information System
GNP	Graphene Nanoplatelet
GNS	Graphene Nanosheet
GO	Graphene Oxide
GQD	Graphene Quantum Dots
GSH	Glutathione
HDL	High-density Lipoprotein
HMG-CoA	3-Hydroxymethylglutaryl Coenzyme A
HPLC	High-Performance Liquid Chromatography
HRSEM	High Resolution Scanning Electron Microscopy
ICM	Iodinated Contrast Media
InP	Indium Phosphate
KTP	Ketoprofen
LC–MS	
LC–MS/MS	Liquid Chromatography Coupled with Tandem Mass Spectrometry
L-Cys	L-cysteine
LDL	Low-density Lipoprotein
LDH	
LOAEL	Lowest Observed Adverse Effect Level
LOD	Limit of Detection
LOQ	Limit of Quantification

MAGA	Methacryloylamidoglutamic Acid
MIP	Molecular Imprinted Polymer
MIP/GNPs/MWNTs/GCE	Molecular Imprinted Polymer/Gold Nanoparticles/Multi-Walled Carbon Nanotube
MIP-NC	Molecular Imprinted Polymer Nanocomposite
MPA	Mercaptoacetic Acid
MPA	Mercaptopropionic Acid
MPA	Mercaptopropyl Acid
MSDS	Material Safety Data Sheet
MTMOS	Methyltrimethoxysilane
MWCNT	Multiwall Carbon Nanotube
NAC	N-Acetyl-L-Cysteine
NOAEL	No-Observed Adverse Effect Level
NOECO	No-Observed Effects Concentration
NSAID	Nonsteroidal Anti-Inflammatory Drug
OA	Oleic Acid
ODE	1-Octadecene
OPH	Organophosphorus Hydrolase
PAH	Polycyclic Aromatic Hydrocarbon
PBS	Phosphate-Buffered Saline
PBT	Persistence, Bioaccumulation and Toxicity
PCB	Polychlorinated Biphenyl
PCP	Personal Care Product
PDMS	Polydimethylsiloxane
p(HEMAGA)	Poly(2-Hydroxyethyl Methacrylate–Methacryloylamidoglutamic Acid)
PL	Photoluminescence
POM	Polyoxometalate
PPCP	Pharmaceutical and Personal Care Product
PTMOS	Phenyltrimethoxysilane
QD	Quantum Dot
QD-MIP	Quantum Dot-based Molecularly Imprinted Polymer
QD-Si	QDs Incorporated into Silica
QSAR	Quantitative Structure–Activity Relationship
rGO	Reduced Graphene Oxide
RSD	Relative Standard Deviation
SANBI	South African National Biodiversity Institute
SiQD	Silicon Quantum Dot
SM	Sulfamethoxazole
SPE	Solid Phase Extraction
SPME	Solid Phase Microextraction
SPR	Surface Plasmon Resonance
SQL	Structured Query Language
SSRI	Selective Serotonin Re-Uptake Inhibitor
SVOC	Semi-Volatile Organic Compound
SWCNT	Single-Walled Carbon Nanotube
SWCNT–GNS/GCE	Single-Wall Carbon Nanotube-Graphene Nanosheet/Glassy Carbon Electrode
SWV	Square Wave Voltammetry
TCC	Triclocarban
TEM	Transmission Electron Microscopy
TEOS	Tetraethoxysilane
TOP	Trioctylphosphine
TOPO	Trioctylphosphine Oxide
TPH	Total Petroleum Hydrocarbon
TRY	L-tryptophan
UA	Uric Acid
USA	United States of America
US EPA	United States Environmental Protection Agency
USGS	United States Geological Survey
UV	Ultraviolet
UV/vis	Ultraviolet Visible
VLDL	Very Low-density Lipoprotein
VOC	Volatile Organic Compound
WRC	Water Research Commission
WRCS	Water Resource Classification System
WWTP	Waste Water Treatment Plant
XRD	X-Ray Diffraction
ZDC	Zinc Diethyldithiocarbamate

1 INTRODUCTION

1.1 Background to the Project

The continuing evolution of science and technology results in numerous advances that influence the lifestyle of humans. These changes have the concomitant effect of altering our interactions with and our impact on our natural environment. As new products, such as pharmaceuticals and consumer items, reach the market and become popular, there is the possibility that levels of contaminants related to these products increase in the environment, which may lead to negative impacts and effects on human and ecosystem health. Many of these contaminants are new and environmental protection mechanisms and technologies are thus not designed to ameliorate their impacts; waste water treatment processes, for example, may not effectively remove them from influent streams.

Emerging chemical pollutants (ECPs) are defined as new chemicals that do not have a regulatory status, but may have an adverse effect on human health and the environment (Liu et al., 2014). Sources and environmental pathways of these ECPs have been increasingly associated with waste and waste waters arising from industrial, agricultural and municipal activities (Gavrilescu et al., 2014). The ECPs of current concern include a wide range of compounds including phthalates, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and bisphenol A, in addition to disinfectants, pharmaceuticals and hormones (Gavrilescu et al., 2014).

There is currently a lack of information regarding the nature, concentration, variability, transport and fate of these compounds in water, which is of global concern (Gavrilescu et al., 2014). In order to understand the potential biological impact of these chemicals, reliable and practical methods to identify and quantify ECPs in water are urgently needed. Analysis of ECPs is complex and challenging due to the wide range of compound classes involved, for which there are no established analytical methods in some cases (Liu et al., 2014). The sample matrix itself is often complex and may interfere with the target analyses and the ECPs are usually present at trace levels in water ($ng{\cdot}L^{-1}$ or $\mu g{\cdot}L^{-1}$).

The analysis of water for ECPs is of vital importance as many of these compounds are known to have adverse human health effects such as endocrine disruption, chronic toxicity, and the development of pathogen resistance to medication (Rosal et al., 2010), which make a holistic understanding of exposure and risk necessary (Daughton, 2004).

Various studies have determined the occurrence and concentrations of pharmaceutical ECPs in effluents from water treatment plants and in rivers in North America, Europe, Asia and Australia. The ECPs fell into the classes of antibiotics, analgesics and anti-inflammatories, anti-epileptics, beta blockers and blood lipid regulators. ECP research in water has also covered psycho-stimulants (such as caffeine); disinfectants (triclosan), phthalates and plasticisers (Deblonde et al., 2011).

Data relating to the occurrence of ECPs in South African waters is currently very limited (Osunmakinde et al., 2013; Patterton, 2013), which can be ascribed in part to the high cost of performing the complex analyses involved. This project addresses this shortcoming by developing novel quantum dot (QD)-based fluorescence sensors for target ECPs of relevance to South Africa. Screening has the advantage over comprehensive methods of allowing for a large sample set to be analysed; thus, trends in time and place can be established. This is possible due to the speed and low cost that such a screening method would present. Samples which screen positive can then be targeted for comprehensive and quantitative analysis such as by LC–MS/MS, if required.

The QDs have several optical properties that make them ideal in this application, which include high quantum yields, high molar extinction coefficients, broad absorption with narrow and symmetric photoluminescence (PL) spectra spanning ultraviolet (UV) to near-infrared, large effective Stokes shifts and high resistance to photobleaching (high photostability). In addition, the fluorescent emission of QDs is size-tunable and they have broad excitation spectra, which allow for excitation at a single wavelength far removed from their emission (Ruedas-Rama et al., 2012). Nanomaterials have been developed for use in a range of water-related applications, including removal of organic pollutants (Salipira et al., 2007), and for the analysis and monitoring of ECPs (Liu et al., 2014). It must be remembered that nanomaterials themselves are considered a class of ECPs, thus their design (core/shell structures) and immobilisation are important considerations to minimise their potential environmental impacts in use.

The novel fluorescence sensors developed in this project have the potential to provide complimentary information to standard analytical approaches and can enable wider monitoring of target ECP contamination in a range of South African waters (including waste water, industrial effluents, surface water and groundwater) in a cost-effective manner, and may also provide information on the effectiveness of waste water treatment processes in the removal of these ECPs. It is thus envisaged that our sensors may assist in enhancing the sound management of our water resources.

1.2 Aims of the Project

The aims of the project were thus to:

1) Prioritise ECPs in water in order to define target analytes for fluorescence screening (based on e.g. toxicity, projected concentrations and fluorescence characteristics) and to identify potential water sampling sites in Gauteng and surrounds.
2) Synthesise and characterise suitable novel QDs and QD nanocomposites for use in the proposed fluorescence sensors.
3) Immobilise the QDs and QD nanocomposites in suitable polymers (such as siloxanes) in order to generate fluorescence sensor prototypes.
4) Test and optimise the fluorescence sensor materials and prototypes using synthetic standard solutions of the target compound(s) in water.

1.3 Contributions of Individual Chapters to the Objectives of the Project

Chapter 2 of this report introduces ECPs including the classes of ECPs, their sources and environmental pathways. Degradation of ECPs in the environment and their removal efficiencies in waste water treatment plants (WWTPs) are also discussed, as is the legislative aspect of ECPs.

In Chapter 3, a method for prioritising ECPs, which were identified as being relevant to South African water bodies, is presented, as well as risk maps identifying areas of greatest concern in the province of Gauteng, which were created by using a geographic information system (GIS).

Chapter 4 covers a literature review of fluorescence- and nanomaterial-based sensors developed to monitor the target organic ECPs in water, which were selected for this proof of concept study [namely, PAHs, pesticides (primarily atrazine), triclosan and acetaminophen]. An introduction to QDs, the synthesis thereof, as well as the principles of optical sensing using these materials are provided.

Chapter 5 details the methods employed in this study to synthesise and characterise CdSe/ZnS and grapheme QDs, while Chapter 6 covers the testing of these fluorescence sensor nanomaterials using standard solutions of the target compounds. Initial results regarding the development of fluorescence sensor prototypes by immobilisation of these nanomaterials into a polymer thin film are included, as is a simple cost benefit analysis to compare the proposed method to standard analytical approaches.

An overall conclusion and suggested future research are provided in Chapter 6.

This project contributed significantly to capacity building and the knowledge gained and generated in conducting this research has resulted in a number of outputs, as detailed in Appendix 2.

2 INTRODUCTION TO EMERGING CHEMICAL POLLUTANTS

By Fritz Petersen[1], James Dabrowski[2] and Patricia Forbes[3]

2.1 Background

Humanity is facing major problems in the twenty-first century related to water quantity and/or water quality issues (Jackson, et al., 2005). The focus on maintaining clean usable water will likely be intensified in the future by climate change, thus resulting in higher water temperatures, melting of glaciers, and an intensification of the water cycle in terms of more intense and more frequent extreme weather events (Huntington, 2006), with potentially more floods and droughts (Oki & Kanae, 2006). A lack of good sanitation, and related to it a lack of safe drinking water, currently affects more than a third of the world population, and therefore is a threat when considering potential human health impacts (Schwarzenbach et al., 2010). An additional related potential threat is exposure to pathogens or to chemical toxicants via the food chain (e.g., the result of irrigating plants with contaminated water or the bioaccumulation of toxic chemicals by aquatic organisms) or during recreation (e.g., swimming in polluted surface water) (Schwarzenbach et al., 2010) as the aquatic environment has been termed the ultimate sink for natural and anthropogenic chemicals (Sumpter, 1998).

Improvement in the fields of science and technology holds many advantages; however, there can be detriments that accompany these improvements as well, especially when considering the negative impacts such advances can have on the environment. Modern science and technology often develop and use new chemicals for the benefit of the human race (such as in the fields of health and agriculture). These chemicals can, however, have unintended effects after they have served their main purpose – especially when they end up in water bodies and other natural ecosystems and start to negatively influence the organisms that live within these habitats. Once these chemicals enter ecosystems they are known as ECPs, which can be defined as new chemicals that do not have a regulatory status, but which may have an adverse effect on human health and the environment (Liu et al., 2014).

There is no standardised definition of what an ECP is as agencies define it differently. It has at times been defined to include regulated chemicals such as PCBs, as PCBs have the potential to cause endocrine disruption, which is why they are currently regulated (Diamond et al., 2011). Other definitions state that ECPs are chemicals that are currently unregulated. This lack of a concise definition means that each study monitors its own subjective list of chemicals (Diamond et al., 2011). This can lead to questions being raised over how efficient the efforts of monitoring agencies are regarding accurately and successfully assessing, and where possible circumventing or mitigating, the negative effects associated with ECPs.

Pharmaceuticals are designed to target specific metabolic and molecular pathways in humans and animals, but they often have important side effects too. This is an example of how a chemical intended to be beneficial might end up causing harm to both the environment and humans. This can occur when ECPs are introduced into the environment and start potentially affecting the metabolic and molecular pathways in animals and other organisms that have identical or similar target organs, tissues, cells or biomolecules (Fent et al., 2006). For example, during the 2000s it was discovered that an unusually high death rate among various species of vulture in Asia was caused by a widely used analgesic and anti-inflammatory drug, namely, diclofenac (Meteyer et al., 2005). Renal failure and visceral gout resulted in population loss as uric acid accumulated throughout the body cavity following kidney malfunction (Meteyer et al., 2005). Residues of diclofenac leading to renal failure were reported by both

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experimental oral exposure and through feeding vultures the diclofenac-treated livestock. Thus, it is apparent that diclofenac shares a direct relationship with renal failure in vultures (Meteyer et al., 2005).

Many ECPs are characterised poorly in terms of their presence in the aquatic environment and their potential effects on aquatic wildlife and humans (Diamond et al., 2011). That being said, acute toxicity for ECPs are unlikely to occur at environmental concentrations, as concentrations at which acute effects occur are usually 100–1000 times higher than residues found in the aquatic environment (Farré, 2008). The only exception to these findings would be in the case of a spill of these chemicals (Fent et al., 2006). Although the reported concentrations of ECPs are generally low, questions have been raised over the potential impacts of these chemicals in the environment on human and animal health after long-term (chronic) exposure (Thomaidis et al., 2012). Thus, the chronic effects of ECPs such as pharmaceuticals are more relevant, because many aquatic species are continually exposed over long periods – even throughout their entire life cycle. However, there has been little information reported to date on different effects and endpoints (Farré, 2008).

In the study of ECPs there is a lack of chronic toxicity data. Even when it is available, the chronic toxicity data is mostly limited to certain species and as such comparison of toxicity between different species becomes difficult. Comparisons between substances are also complicated as the toxicity of the substances is not always tested on the same organisms. Studies on the chronic toxicity often do not investigate the important key targets; nor do they address the question of different organisms (Fent et al., 2006). Toxicity experiments are usually performed according to established guidelines whereas specific investigations including analysis of possible targets of various ECPs, or over the different life stages of the aquatic organisms, are lacking or extremely rare (Fent et al., 2006). Life cycle analyses have largely only been reported for ethinylestradiol (EE2) (Parrott & Blunt, 2005) and toxicity to benthic and soil organisms have very rarely been evaluated (Fent et al., 2006).

Acute toxicity is the main focus of the available literature on the ecotoxicological effects of ECPs and it is generally focused on aquatic organisms (Fent et al., 2006). The effects of environmental parameters, such as pH, on the toxicity of ECPs have only been rarely explored. Studies such as the aforementioned would be of importance when, for example, one considers the case where ambient pH can induce different toxicities for acidic pharmaceuticals (Fent et al., 2006).

It should be noted that ECPs can undergo chemical transformations in the environment and can thus have varying properties (Farré, 2008). This largely depends on the compartment in which the ECPs are present in the environment (such as groundwater, surface water or sediment) or in the technosphere (such as WWTPs and drinking water facilities). Diverse transformations can take place, sometimes producing products that can differ in their environmental behaviour and ecotoxicological profile (Farré, 2008). For example, transformation products of some pollutants are often more persistent than their corresponding parent compounds or exhibit greater toxicity (for example, the major biodegradation product of nonylphenol ethoxylates, nonylphenol, which is much more persistent than the parent compound and can mimic oestrogenic properties) (Boxall et al., 2004).

A biologically active compound’s effect is typically the result of an interaction with a receptor and the particular moiety of the molecule (Boxall et al., 2004). If the active moiety remains intact during degradation, then the degradate may have the same mode of action as the parent (Boxall et al., 2004). There is also, however, the possibility that a transformation process could occur, which can result in a degradate that has a different and more potent mode of action than the parent. This means that the degradate will likely be more toxic than the parent (Boxall et al., 2004). The effects of drug metabolites and breakdown products have also rarely been investigated; however, the relevance of studying compounds can be seen if the phototransformation products of naproxen are considered. This is because they showed higher toxicities than the parent compound, though genotoxicity was not found (Isidori et al., 2005).

After being introduced into the environment, degradates may be transported and distributed between the major environmental compartments (Fent et al., 2006). The concentrations in these compartments can vary greatly depending on numerous factors and processes, including how the parent compound is released to the environment; how fast it degrades; the half-lives of the degradates; partitioning to sludge, soil, and sediment; and subsequent movement to air and water (Fent et al., 2006).

2.2 Emerging Chemical Pollutants in a South African Context

Water pollution and the potential problems associated with ECPs are of particular concern to South Africa as it is a water scarce country where only 8.6% of the rainfall is available as surface water (Haarhoff et al., 2015). South Africa's freshwater resources are already being used to capacity. The amount of available freshwater could become a limiting resource in determining the success of the goal of continued socio-economic development in the future (Haarhoff et al., 2015).

A large proportion of South Africa's population live in rural or poorly developed areas with inadequate infrastructure, which includes a diminished water value chain (Ncube et al., 2012). This leads to undesirable scenarios and increases the risk of these people either contaminating the water or relying on and using water that has already been contaminated. In these areas, there can be either no formal treatment or distribution of the water resource, which leads to consumers taking their drinking water directly from the water resource (Haarhoff et al., 2015). These areas can also suffer from the lack of a protected distribution system to direct treated water from the source to a tap in the homes of the consumers. This leads to scenarios where the inhabitants of these areas are forced to carry water in buckets (at times from unsanitary sources) to be used and stored in their homes. This form of water transport has been referred to as the "human pipe" (Ncube et al., 2012).

Transporting water in this manner (via a human pipe) can entail many hazards for the people who use and consume this water in their daily lives. In addition to the pollution (both ECPs and conventional pollution) that was initially in the water, the quality of the water may be further diminished by the inappropriate use of materials for buckets (Haarhoff et al., 2015). These buckets can be plastic cans or poorly cleaned containers that contain biofilms on the inside. Lack of formal schooling and education in these regions also increase the risk of water recontamination occurring in the homes of consumers. In areas where there is this described lack of formal water infrastructure and there is a decreased water value chain, the ECPs and all pollutant concentrations in the raw water should ideally be strictly monitored and controlled to minimise the potential damage these substances can cause (Haarhoff et al., 2015).

The rapid rate of industrialisation coupled with population growth and climate change have led to an increase in the awareness of the harm that humans can do to the environment. As such there is a growing cognizance of the accumulative amounts of chemicals being discharged into the environment from waste water treatment effluents as well as industrial and agricultural sources.

South Africa has approximately 930 water supply systems; thus, sampling and analysing all these systems regularly for the thousands of potentially harmful chemicals would not only be extremely costly, it would also be very time-consuming (Haarhoff et al., 2015). This is a problem faced by all policymakers and regulatory bodies around the world, but it cannot be used as an excuse for inaction. A possible solution to the problem can be found by implementing GIS as a tool to reduce the amount of sampling sites by determining hotspots based on relevant variables. These sampling hotspots can then be sampled regularly to determine the health of the water body within which it is located, and it can also be used to test for the presence of specific ECPs in the aquatic system.

Sampling for ECPs in South Africa usually only occurs at sites located within areas that are perceived to have high levels of pollution. It is worth noting that even with this being the case, there are few instances where concentrations are found to be high enough to cause concern (Haarhoff et al., 2015). However, there is a distinct lack of information on the current status of ECPs and ECP contamination in South Africa. Although the National Toxicity Monitoring Program is available, its efficiency is diminished due to limited sampling sites, analysis and budget (Haarhoff et al., 2015). Another problem in South Africa is the lack of periodic and ongoing data collection. This is especially important as South Africa is a developing country (Haarhoff et al., 2015).

The World Health Organization has stressed the importance of gaining more knowledge and data on ECPs. Six aspects have been highlighted as the most important regarding ECPs and the influence that ECPs can have on both the environment and humans. These six aspects are (Bergman et al., 2013):

1) Strengthening knowledge of ECPs particularly in developing countries where there is a lack of data.
2) Enhancing testing (mixtures and effects).
3) Reducing exposure and susceptibility to disease.
4) Identifying endocrine-disrupting chemicals (EDCs).
5) Promoting scientific advances, innovation and disease prevention by creating suitable conditions for this to occur.
6) Finding clear methods to assess the strength of the evidence between exposures to chemicals and adverse health effects.

A workshop on ECPs was held in Pretoria in 1999, where the need for reliable and relevant data on ECPs and especially EDCs in South African water systems was expressed. Consequently, the Water Research Commission (WRC) initiated a research programme on EDCs in 2001. This led to the development and publication of a strategic research plan by the WRC in 2005 (Burger & Nel, 2005). This plan included the compilation of a list of priority compounds (for effects on both humans and animals).

More recent studies on ECPs found evidence of these compounds in one of Gauteng's largest water bodies, namely, the Rietvlei Dam and the adjoining Rietvlei Nature Reserve (Barnhoorn et al., 2011). Evidence of intersex fish and wildlife with calcified testes was found. The authors linked this with elevated levels of lindane, dichloro-diphenyl-trichloroethane (DDT) and PCBs found in the fatty tissues of the organisms. These findings indicated that ECPs can be present in a multitude of environments and as such they cannot be ignored.

South Africa has a large agricultural sector that is also related to certain ECPs due to the use of several herbicides, insecticides, nematocides and fungicides on crops, as well as substances that stimulate growth, and veterinary products in animal husbandry (Haarhoff et al., 2015). The detection, monitoring, control and treatment of these substances are more complex than other substances, because these chemicals are non-point pollutants (Haarhoff et al., 2015).

2.3 Classes of Emerging Chemical Pollutants

The ECPs can be categorised into groups of substances: pesticides, pharmaceuticals [including analgesics and non-steroidal anti-inflammatory drugs (NSAIDs)], antibiotics, beta blockers, blood lipid lowering agents, neuroactive compounds, endocrine-disrupting hormones, steroids, diuretics, psycho-stimulants and antidepressants), contrast media, personal care products (PCPs) (including disinfectants and phthalates), PAHs and flame retardants (Fent et al., 2006).

These substances are briefly discussed to highlight their beneficial uses, the hazards they might pose and their primary characteristics. Many inorganic ECPs have been mentioned in the literature (for example certain heavy metals and metalloids), but this dissertation focuses on organic ECPs.

2.3.1 Pesticides

The importance of agriculture in Africa, and because South Africa is the highest user of pesticides in sub-Saharan Africa (Dalvie et al., 2009), means that pesticides have to be considered when discussing and prioritising ECPs in a South African context. Pesticides were included in this study because there are, as previously mentioned, multiple definitions for the term “emerging chemical pollutant”. These definitions can at times include or exclude pesticides based on the author’s discretion. The decision to include pesticides in this study was ultimately made to incorporate as many substances as possible and was also influenced by the size of the agricultural sector of South Africa.

The uninterrupted use of pesticides can result in damage to the environment, human toxicity, reduced agricultural production, and reduced agricultural sustainability. Both plants and animals can be adversely affected by numerous short- and long-term effects already on record, including human deaths (Wilson & Tisdell, 2001). Pesticides can cause numerous health problems in humans including chronic neurotoxicity, endocrine disruption, immune impacts, genotoxicity, mutagenicity and carcinogenesis (Dabrowski et al., 2014).

Pesticides can move throughout the environment via run-off, leaching and spray drift (Schulz, 2001) causing pesticides to occur in non-target environments, especially in ground- and surface water resources (Dabrowski et al., 2002). The amounts of pesticides that can be transferred to surface waters from run-off are reliant on the time interval between the application of the pesticides and the first heavy rainfall event, the slope and soil types of the catchment, the quantity of applied pesticide, the chemical nature of the pesticide, and the size and characteristics of buffer strips (Dabrowski et al., 2002).

The unintended devastation of vital agricultural predators of pests as a result of pesticide use has led to the spread of several pests and diseases (Wilson & Tisdell, 2001). Initially the use of pesticides was effective in reducing pest infestations and in increasing agricultural production and productivity. However, over time targeted pests developed resistance to pesticides, thus necessitating increasing applications, which resulted in rising populations of pests, or both (Wilson & Tisdell, 2001). After a point, resistance of pests can grow to such an extent that the application of pesticides is no longer an economically viable solution. Once application stops, the population of pests may climb to levels in excess of those pre-dating the use of pesticides. They may remain permanently above levels prior to using pesticides. This can occur as a direct consequence of the pesticides eliminating the beneficial predators of pests (Wilson & Tisdell, 2001). Pesticides are among several proposed causes for the decline in amphibian populations globally (Hayes et al., 2006). In South Africa, atrazine, mancozeb and acetochlor have been ranked as the top three priority pesticides in terms of their persistence, toxicity and usage information (Dabrowski et al., 2014).

Malaria is a disease that kills many people each year. DDT, a pesticide, is used in effort to control the threat posed by the malaria-carrying mosquito (Bornman et al., 2010). The efficacy that DDT has shown in decreasing cases of malaria as well as malaria-related deaths when sprayed indoors has led to its continued use in efforts to control malaria (Bornman et al., 2010). The Stockholm Convention has granted South Africa restricted use of DDT for indoor residual spraying even though the use of DDT has been banned internationally (Bornman et al., 2010). Not only is DDT harmful towards the environment due to its toxicity, but it is also an EDC (Bornman et al., 2010).

The acute toxicity of DDT within most mammals is low; however, very limited information is available on the effect that chronic exposure (sufficient to cause hormone disruption) has on humans and animals (Bornman et al., 2009). This is especially true in South Africa as studies investigating the effect that chronic low dose exposure of DDT in the environment will have on aquatic as well as human health are severely lacking (Bornman et al., 2009). Bornman et al. (2009) reported that residues of DDT have been found in fish fat samples taken from South African water bodies, thus it is known that DDT does occur in South African water bodies, which warrants further investigation.

A South African study (De Jager et al., 2009) reported a correlation between DDT and dichloro-diphenyl-dichloroethylene (DDE) and the occurrence of sperm with chromatin defects in young men. The disruption of normal hormone function and DDT/DDE has widely been reported to cause adverse health effects.

2.3.2 Pharmaceuticals

2.3.2.1 Non-steroidal anti-inflammatory drugs

Drugs such as NSAIDs are commonly used to treat inflammation, treat pain and relieve fever. Sometimes they are also used for long-term treatment of rheumatic diseases (Fent et al., 2006). The NSAIDs act by inhibiting either reversibly or irreversibly one or both of the two isoforms of the cyclo-oxygenase enzyme (COX-1 and COX-2), which catalyse the synthesis of different prostaglandins (active lipid compounds that cause hormone-like effects in mammals) from arachidonic acid (Vane & Botting, 1998). Since NSAIDs inhibit non-specific prostaglandin synthesis, most side effects after long-term treatment are related to the physiological function of prostaglandins (Fent et al., 2006). Renal (kidney) damage and renal failure after chronic NSAID treatment have been known to occur and seems to be triggered by the lack of prostaglandins in vasodilation-induction (Fent et al., 2006).

Paracetamol (acetaminophen) is a popular over-the-counter pain reliever and fever reducer. Paracetamol’s mode of action is not yet fully understood, but it is thought that this drug acts mainly by inhibiting the cyclooxygenase of the central nervous system (CNS) (Fent et al., 2006). The lack of inhibition of peripheral cyclooxygenase involved in inflammatory processes is the reason why paracetamol is thought to lack anti-inflammatory effects (Fent et al., 2006). Adverse effects of paracetamol are mainly due to formation of hepatotoxic metabolites, primarily *N*-acetyl-*p*-benzoquinone imine, which are synthesised when the availability of glutathione (GSH) is diminished in liver cells (Fent et al., 2006).

2.3.2.2 Antibiotics

Following penicillin’s discovery in 1928 by Sir Alexander Fleming, antibiotic use in human and veterinary medicine has become a common therapeutic practice that has led to both high consumption and the gradual accumulation of antibiotics in the environment (Manzetti & Ghisi, 2014). Antibiotic contamination can have a multitude of sources including waste water, landfills, urban sites, and industrial and hospital effluents (Renew & Huang, 2004; Watkinson et al., 2009). Antibiotic compounds are largely composed of structures encompassed by cyclic components, represented by benzene rings, piperazine units, hexahydropyrimidines, and sulphonamides, quinolone and morpholine groups (Renew & Huang, 2004). These compounds have metastable properties and yield both activated metabolites, conjugates and hydroxylated forms after being metabolised by humans and animals (García-Galán et al., 2008). These characteristics of antibiotics mean that when they are continuously released into the environment, a range of diverse active chemical compounds with possible reactive properties and largely unknown consequences enter the environment. It should also be mentioned that there is a lack of data on the potential increased resistance of clinically relevant microbes found in water bodies, which is associated with antibiotic contamination of these water bodies (Cizmas et al., 2015).

Antibiotics can enter the environment in a variety of ways, from their production (direct discharges as a result of production can be extremely high) as active pharmaceutical ingredients, through excreting residues after usage or by discarding unused medicines (Sarmah et al., 2006). Considerable amounts of active residues can be found in the urine and faeces of individuals who use antibiotics. Waste waters can thus be rich in antibiotic residues; however, they have also been found in marine environments (Kümmerer, 2009). The degradation rates of different antibiotics in the environment can be vastly different. For example, penicillins are easily degraded whereas fluoroquinolones and tetracyclines are much more persistent, which means that they will have a greater capacity to accumulate in the environment (Larsson, 2014). It should, however, be noted that only a fraction of the total amounts probably remains bioactive as many antibiotics tend to bind strongly to particles. The precise amount of bioavailable fractions of antibiotics in solid matrices is an important consideration to assess risks, but accurately determining this is still a major challenge (Boxall et al., 2012).

Antibiotics can be biologically converted to solubilized forms, including glucuronated, GSH-conjugated and possibly arachidonated conjugates, as well as hydroxylated and nitro-reduced forms (Farkas et al., 2007). Some of these forms have an increased capacity to be assimilated and accumulated in the environment (Farkas et al., 2007) and are therefore potentially absorbed in more advanced species, such as fish, animals and humans through nutrition and diet. This has recently been experienced where chloramphenicol glucuronic conjugates have been found in the tissues of poultry, in bee honey, and shrimp from Asian countries (Ferguson et al., 2005). Most species, particularly vertebrates, feature pathways and systems that metabolically break down xenobiotics and exogenous molecules in similar manners (Thomas, 2007). The chemical properties, functional groups and reactive atoms in the structures of antibiotics determine their metabolic fate. Thus, diverse types of antibiotics can be treated differently by the body and, therefore, precede different types of metabolite in the excretion (urine/ faeces) (Manzetti & Ghisi, 2014).

Metabolites of antibiotics are sources of pollution in the environment; particularly if thought is given to the water compartment and groundwater reserves. With the ever-growing use of antibiotics and the poor regulation of their metabolites in the environment, the accumulation of antibiotics and their metabolites in animals and humans can thus be considered an environmental and toxicological threat. An estimated 100 000–200 000 tons of antibiotics are consumed each year. Large quantities of their residues are released into the environment that feed the cycle of biotransformation and bioaccumulation of antibiotics in the environment (Kümmerer, 2009). Waste waters are major carriers of antibiotics in heavily populated areas. Therefore, the effective decontamination of waste waters is critical for water purification and avoidance of their accumulation in the environment (Hu, 2013). It is worth noting that antibiotics have also been reported in drinking water (Schaider et al., 2014).

Antibiotics are a group of ECPs that require constant monitoring in the environment with particular focus on heavily populated urban areas (Manzetti & Ghisi, 2014). Ideally, antibiotics should not affect humans and should only be toxic to bacteria and microbes; however; reality is more complicated, as several classes of antibiotics used for therapy have direct toxic effects at prescribed doses (Larsson, 2014). Research indicate that metabolites of antibiotics can be persistent and accumulate in food and drinking supplies, including groundwater (which was originally thought to be resistant to substantial contamination) (Manzetti & Ghisi, 2014). As previously mentioned, waste water is the most prominent environmental compartment impacted by these contaminants, and modern decontamination approaches within WWTPs do not remove the antibiotic compounds fully, resulting in a low but never diminishing concentration remaining present in the environment (Manzetti & Ghisi, 2014).

In a study by Martinez Bueno et al. (2012), ECPs in waste water were typically detected in the range of a few $ng{\cdot}L^{-1}$ to a few hundred $ng{\cdot}L^{-1}$, although there were some exceptions to this as more frequently used compounds were detected in the $mg{\cdot}L^{-1}$ range. These ECPs included atenolol, gemfibrozil, galaxolide, caffeine, acetaminophen, diclofenac, ofloxacin, ibuprofen, codeine, naproxen, paraxanthine and fenofibric acid (Bueno et al., 2012). Antibiotics, analgesics and anti-inflammatories were found most commonly, and typically had the highest detection ranges as compounds classes (Bueno et al., 2012).

2.3.2.3 Beta blockers

Beta blockers typically act by competitively inhibiting beta-adrenergic receptors with the purpose of lowering high blood pressure (hypertension), and circumventing the repetition of heart attacks in people who have already experienced one (Fent et al., 2006). Depending on the medical needs of a patient, beta blockers may selectively inhibit one or multiple receptor types, where this selectivity is based on dissimilarity in chemical groups added to compounds that are able to enhance the interactions with amino acids of the trans membrane domains (Fent et al., 2006). Some beta blockers (e.g. propranolol) can cause cell membrane stabilisation, while others (e.g. metoprolol) have no membrane-stabilising qualities (Doggrell, 1990). Side effects of beta blockers are mainly bronchoconstriction and disrupted peripheral circulation.

2.3.2.4 Antilipidemic drugs

Antilipidemic drugs (which promote the reduction of lipid levels in the blood) can be divided into two groups, namely, statins and fibrates. Fibrates are detected in the aquatic environment more often than statins (Fent et al., 2006). Both types act within the blood plasma to decrease the concentration of cholesterol (statins and fibrates) and triglycerides (fibrates) (Fent et al., 2006). Statins inhibit cholesterol synthesis by impeding the 3-hydroxymethylglutaryl coenzyme A (HMG-CoA), which plays a vital part in limiting cholesterol synthesis during the step where HMG-CoA is converted to mevalonate (Laufs & Liao, 1998). The resorption of low-density lipoprotein (LDL) cholesterol from blood plasma then takes place as a result of the intracellular cholesterol depletion that occurs when the expression of LDL receptors in hepatocyte membranes is increased (Fent et al., 2006). Fibrates likely act by activating the lipoprotein lipase enzyme, which is mainly responsible for converting very low-density lipoprotein (VLDL) to high-density lipoprotein (HDL), which will therefore lead to a decrease in plasma triglycerides concentration (Staels et al., 1998).

Antilipidemic drugs, like most drugs, can also pose certain risks to the health of organisms. Fibrates stimulate cellular fatty acid uptake, conversion to acetyl-CoA derivatives, and catabolism by the beta-oxidation pathways, which, combined with a reduction in fatty acid and triglyceride synthesis, result in a decrease in VLDL production (Staels et al., 1998). Hepatic damage may occur after chronic exposure to fibrates (Qu et al., 2001) and this is thought to be related to inhibition of mitochondrial oxidative phosphorylation (Keller et al., 1992). Furthermore, fibrates can cause a massive proliferation of peroxisomes. There is a strong correlation between fibrate exposure and hepatocarcinogenicity in rodents; however, this was not the case in humans (Cajaraville et al., 2003).

2.3.2.5 Neuroactive compounds

Anti-epileptic drugs act on the CNS by reducing the overall neuronal activity (Fent et al., 2006). This can be achieved in two ways, namely, the blocking of voltage-dependent sodium channels of excitatory neurons (e.g. carbamazepine), or by intensifying the inhibitory effects of gamma-aminobutyric acid (GABA) neurotransmitter (which is an amino acid that acts as a neurotransmitter in the CNS) by binding on a specific site in the gamma subunit of the corresponding receptor (e.g. diazepam) (Rogers et al., 1994).

Several studies have found that antidepressants and anxiolytic drugs alter the behaviour of fish, molluscs and crustaceans even at extremely low concentrations (Fong & Ford, 2013). The intended targets of antidepressants, anxiolytic and neuropathic drugs, for example, terephthalic acid [a selective serotonin re-uptake inhibitors (SSRI)], blocking voltage-gated sodium channels and GABA agonists, and specific antihypertensive compounds, are highly maintained across vertebrates; 61% are also found in the invertebrate crustacean *Daphnia* (Gunnarsson 2006; Rivetti et al., 2015). It then follows that neuroactive drugs may adversely affect aquatic invertebrates.

Several neuroactive compounds are intended to affect neurotransmitters (for example, serotonin, dopamine, epinephrine, GABA, which control multiple physiological and behavioural processes) (Fong & Ford, 2013). The tendency of antidepressants at low concentrations to not follow a monotonic responses is increasingly being reported (Rivetti et al., 2015).

Similar findings have been reported for both endocrine and neuroactive compounds. While they are present at low concentrations, they act specifically on their target sites; however, if they are present at high concentrations, they become toxic and impair the survival, growth and reproduction of organisms, regardless of its original intended purpose (Rivetti et al., 2015). There is thus a necessity to study the continuous effects of neuroactive compounds at varying concentrations levels in non-target organisms.

The SSRIs function by blocking the re-uptake of serotonin in the nerve synapses and are prescribed universally to treat clinical depression in humans (Rivetti et al., 2015), which leads to these compounds becoming continuously more prevalent throughout the environment.

It was recently theorised that increased levels of synaptic serotonin found in water bodies was caused by SSRI treatment that increased post-synaptic neuronal activity in *D. magna*, which alters the organism's view of the food environment and switches its life history responses towards those usually only found during times of the highest levels of food availability (Rivetti et al., 2015). In invertebrates, SSRIs can thus affect serotonin transporters, serotonin and other receptors. This can then negatively affect numerous organisms including, for example, crustaceans whose serotonin regulates neuro-secretory organs that release neurohormones that control reproduction, growth, maturation, immune function, metabolism, behaviour and colour physiology (Fong & Ford, 2013).

Diazepam (Valium) is generally used to treat anxiety as it augments the effect of the neurotransmitter GABA. It does so by binding to the benzodiazepine site on the GABA receptor, which leads to a depression of the CNS (Rivetti et al., 2015).

Carbamazepine is usually prescribed to treat epilepsy and neuropathic pain (Rivetti et al., 2015). It achieves this by stabilising the inactivated state of voltage-gated sodium channels, which reduces the number of these channels that are available to open, thus leaving the targeted cells less reactive until the drug dissociates (Ambrósio et al., 2002). Carbamazepine is moderately persistent in water and can be found at concentrations ranging from 1 $ng \cdot L^{-1}$ to 3000 $ng \cdot L^{-1}$ in rivers receiving waste water treatment effluents (Muñoz et al., 2009). It was reported that carbamazepine decreased population growth rates in *Daphnia* at 200 $\mu g \cdot L^{-1}$. Propranolol and fluoxetine impaired reproduction at 110 $\mu g \cdot L^{-1}$ and 125 $\mu g \cdot L^{-1}$, respectively (Dzialowski et al., 2006; Hansen et al., 2008).

Carbamazepine and diazepam can start affecting phenotypic responses (e.g. behaviour) shortly after an organism is exposed to them, whereas a different neuroactive compound such as fluoxetine required at least one week to induce the same response (Rivetti et al., 2015). Certain neuroactive compounds can alter reproduction by delaying the time of the first reproduction. As such, it will negatively affect population growth rates (Rivetti et al., 2015). This contributes to the potential risk that these substances can pose to the environment.

2.3.2.6 Endocrine-disrupting chemicals

An EDC is an exogenous substance or mixture that alters the function of the endocrine system and therefore causes negative effects in an intact organism, its offspring, or subpopulations (Mills & Chichester, 2005). Numerous classes of chemical show endocrine-disrupting properties. The EDCs have the potential to interfere with normal reproduction and development, which are controlled by an array of hormonal signals in a number of ways. This includes mimicking endogenous hormones, antagonizing normal hormones, altering the natural pattern of hormone synthesis or metabolism, or modifying hormone receptor levels (Sonnenschein & Soto, 1998).

When interference by exogenous substances occurs to the internal endocrine signalling pathways of an organism, endocrine disruption has occurred (Cheek et al., 1998). The similarity of the reproductive physiology of mammalian and non-mammalian vertebrates in so far as the broad structure and function of the reproductive axis involving the hypothalamus, pituitary and gonads is concerned, lead to the fact that many different species are susceptible to EDCs Mills & Chichester, 2005). Furthermore, EDCs can impact organisms living in various aquatic environments as they have been found in freshwater, estuarine, and marine environments (Mills & Chichester, 2005). Interference with the normal synthesis, storage, release, transport, metabolism, binding, action or elimination of endogenous hormones is how EDCs are likely to influence these susceptible organisms (Kavlock et al., 1996). It is worth mentioning that anti-oestrogenic activity has even been displayed by several PAHs in a yeast-based oestrogen receptor binding assay (Tran et al., 1996).

Evidence for endocrine disruption from the natural environment has been commonly reported in studies that focus on animal species that either live in water or are very water dependent (Aneck-Hahn et al., 2009). Thus, the aquatic environment generally forms the basis for endocrine disruption studies and sampling. The vulnerability of aquatic ecosystems is further incited by the fact that most EDCs and their breakdown products will inevitably end up in an aquatic ecosystem somewhere in the world (Aneck-Hahn et al., 2009). These aquatic ecosystems can simply be the habitat of various organisms or it can be used as a drinking source for humans.

Evidence for endocrine disruption in the form of intersex fish has been reported in both freshwater and marine environments (Aneck-Hahn et al., 2007). Male rats dosed with ecologically applicable concentrations of p-nonylphenol resulted in harmful effects on both the Sertoli cells and the testes (Aneck-Hahn et al., 2009). The pesticide DDT is still used in certain South African areas (such as in the Limpopo Province) as a malaria vector control. It was discovered that non-occupational exposure to DDT damaged the semen of human males (Aneck-Hahn et al., 2007). The threat of EDCs in South Africa has not been avoided or mitigated, and thus is similar to other countries of the world in that both animals as well as humans are at risk (Aneck-Hahn et al., 2009). The threat that EDCs pose to the South African environment and to human health in general is thought to be more severe and widespread than originally predicted. Thus, there is a dire need to inform the general public about the risk. Furthermore, further scientific study is required, especially to gather more epidemiological information (Aneck-Hahn et al., 2007).

2.3.2.7 Antiretrovirals

The use of ARVs per capita in South Africa is elevated and higher than any other nation due to the number of people living with HIV/Aids in this country (Wood et al., 2015). It has been reported that approximately 2 150 880 people living in South Africa were receiving ARV treatment in 2012 in contrast to the estimated 199 000 people who were treated in Eastern Europe (World Health Organization et al., 2013a). As such, these compounds should be considered as emerging pollutants, especially in South Africa.

The greater number of people who use and rely on ARV treatment in South Africa have been speculated to possibly lead to an increased number of these compounds that end up in the environment (Wood et al., 2015). This could lead to a unique and possibly detrimental scenario regarding the presence and transformation of these compounds once they enter the environment. The potential impact of these compounds on the environment and the water resources of the country can be aggravated by the overall low rainfall and water scarcity in sub-Saharan Africa; which would mean that there will be little or no dilution of these target compounds that can occur naturally in the environment. The sheer number of compounds used for treating HIV/Aids and thus the breadth of the compound class also present analytical challenges (Peng et al., 2014). These compounds include the following: nucleoside and non-nucleoside reverse transcriptase inhibitors, protease inhibitors, fusion inhibitors, entry inhibitors and integrase strand transfer inhibitors, which are used to treat HIV (Peng et al., 2014) and prevent mother-to-child transmission (Mofenson, 2010). Compounds include Zalcitabine, Tenofovir, Abacavir, Efavirenz, Lamivudine, Didanosine, Stavudine, Zidovudine, Nevirapine, Indinavir, Ritonavir and Lopinavir (Wood et al., 2015; Peng et al., 2014).

Unlike with substances such as antibiotics, which promote drug resistance in the naturally occurring microbes in the environment, the consequences, impacts and environmental relevance of ARVs in water supplies might not be apparent immediately (Peng et al., 2014). HIV does not have a non-human host and cannot occur in the environment as is the case with other viruses or bacteria. The model for the transfer of resistant genes between species is also not relevant and cannot be applied when considering the nature and characteristics of this virus (Wood et al., 2015). The effects that ARVs can have on other environmental retroviruses is largely unknown and literature on the topic is lacking (Peng et al., 2014; Wood et al., 2015). It should be mentioned that even if the presence of these compounds in the environment does not affect the target virus, they could still stimulate the development of drug resistance in other pathogens (Peng et al., 2014; Wood et al., 2015).

2.3.3 Personal care products

Due to the direct route that PCPs follow from being discharged into domestic waste water, which travels from residential areas into the water resources and passes through WWTPs, they form an essential group of compounds to consider (Haarhoff et al., 2015). PCPs are typically also used in considerable quantities and their potential to cause significant damage to both human beings and the environment is greatly enhanced.

PCPs are a varied group of compounds generally used to make products such as soaps, lotions, toothpaste, fragrances, and sunscreens (base) (Brausch & Rand, 2011). The primary classes of PCPs include disinfectants (e.g. triclosan), fragrances (e.g. musk), insect repellents [e.g. *N*,*N*-diethyl-*meta*-toluamide (DEET)], preservatives (e.g. parabens) and UV filters (e.g. methylbenzylidene camphor) (Brausch & Rand, 2011). PCPs are products intended for external use on the human body and thus are not subjected to metabolic alterations (which pharmaceuticals are subject to); therefore, large amounts of PCPs move into the environment unchanged and in their unaltered forms (Ternes & Hansruedi, 2004). Many PCPs are used in large quantities and many have been found to be environmentally persistent, bioactive, and have the potential for bioaccumulation (Peck, 2006).

PCPs comprise some of the most commonly detected compounds in surface water throughout the world (Peck, 2006); however, unlike the case with pharmaceuticals, there is a lack of knowledge on PCP toxicity (Daughton & Ternes, 1999). Multiple studies have been published on the topic of pharmaceutical occurrence and toxicity (Jorgensen & Halling-Sorensen, 2000; Crane et al., 2006; Fent et al., 2006), but a significantly smaller number of studies have determined the potential risk that releasing PCPs into aquatic environments pose. However, when reported, the risk posed by PCPs in water bodies to human health has commonly been reported as being inconsequential (Cizmas et al., 2015).

2.3.3.1 Triclosan and triclocarban

Triclosan and triclocarban are used in soaps, deodorants, skin creams, toothpaste and plastics as antimicrobials (McAvoy et al., 2002). Triclosan and triclocarban are among the 10 most regularly detected organic waste water compounds when considering both frequency and concentration (Brausch & Rand, 2011). The methyl derivative methyl triclosan, which is usually found in WWTP effluent, is relatively stable and lipophilic and as such is likely to bioaccumulate in biota (Brausch & Rand, 2011). Triclosan already has known ecological effects, for example, it has been found to induce alterations in swimming performance of fish such as *Oncorhynchus mykiss, Danio rerio*, and *Oryzias latipes* at low concentrations (71 $\mu g \cdot L^{-1}$). These measured concentrations are considerably greater than other endpoints indicating that this behaviour is not a sensitive endpoint for identifying triclosan effects (Orvos et al., 2002). It is worth mentioning that triclosan is also weakly oestrogenic; exposure to triclosan has been associated in changes in fin length and sex ratios of medaka fish (*O. latipes*) (Brausch & Rand, 2011). These oestrogenic effects have been attributed to its similarities in structure to the non-steroidal oestrogen diethylstilbestrol (Ishibashi et al., 2004).

2.3.3.2 Fragrances

The most widely studied class of PCPs are possibly fragrances as they are suspected of being nearly omnipresent contaminants in the environment (Daughton & Ternes, 1999). Synthetic musk is the most commonly used fragrance and is used in a wide range of products including deodorants, soaps and detergents (Brausch & Rand, 2011). Synthetic musk can be divided into two classes, namely, nitro musk (introduced in the late 1800s) or polycyclic musk (introduced in the 1950s) (Daughton & Ternes, 1999). Active efforts are underway to phase out nitro musk due to its environmental persistence and potential toxicity to aquatic species (Daughton & Ternes, 1999). This means that polycyclic musk is currently used in higher quantities with especially celestolide, galaxolide and toxalide seeing widespread use.

Both types of musk are water soluble, but the typical high octanol-water coefficient common to polycyclic musk (Schramm et al., 1996) indicates the high possibly for these musks bioaccumulating in aquatic species (Brausch & Rand, 2011). This potential has been confirmed by numerous studies that found high concentrations of musk in lipids from fresh- and saltwater fish and molluscs (Schramm et al., 1996).

Nitro musk has a negligible propensity to cause acute toxicity to aquatic taxa studied to date. It has been suggested that nitro musk transformation products possess the potential to be highly toxic to aquatic organisms although there is little data to support this (Daughton & Ternes, 1999). Polycyclic musk is more acutely toxic than nitro musk based on published literature (Brausch & Rand, 2011). However, limited research has been conducted on the effects of musk on algae and benthic invertebrates, and therefore the potential risk cannot be determined accurately (Brausch & Rand, 2011).

Synthetic musk has a high octanol-water coefficient, and as such benthic invertebrates are suspected to be exposed to high concentrations of synthetic musk located within the sediment and ought to be tested to evaluate the potential toxicity of musk released in WWTP effluent (Brausch & Rand, 2011). Very few studies have scrutinized synthetic musk toxicity to sediment/soil organisms although there is a potential risk of exposure to musk for benthic invertebrates (Brausch & Rand, 2011).

2.3.3.3 N,N-diethyl-m-toluamide (DEET)

DEET is the most common active ingredient in insect repellents (Costanzo et al., 2007) and is consistently identified in surface waters (Brausch & Rand, 2011). This insecticide was developed during the 1940s and its method of action relies upon interfering with insects' capability to detect lactic acid on their hosts. DEET is currently registered for use in 225 products. It is estimated annual usage exceeds 1.8 million kg in the United States of America (USA) alone (US EPA, 1998). DEET is moderately persistent in the aquatic environment; however, unlike the case with many other PCPs (e.g. fragrances), DEET has a low bioconcentration factor and is not likely accumulated into aquatic organisms (Costanzo et al., 2007). There is currently a lack of data pertaining to acute toxicity of DEET to aquatic organisms (Brausch & Rand, 2011).

Studies found that DEET is only marginally toxic to aquatic organisms (Brausch & Rand, 2011). Even though DEET is fairly resistant to breakdown and is commonly found in surface water, no studies could be found that specifically examined the chronic toxicity of DEET to aquatic organisms. DEET has been found to impede cholinesterase in rats; however, DEET had no effect on sperm count, morphology, or viability in male rats after a 9-week exposure (Brausch & Rand, 2011).

Costanzo et al. (2007) performed a preliminary risk assessment based on available data and concluded that DEET is unlikely to produce biological effects at environmentally relevant concentrations in aquatic ecosystems. Due to the lack of chronic toxicity information, a definitive assessment could not be made. Analogous conclusions surrounding the biological risk posed by DEET are still pertinent while the chronic toxicity of DEET to aquatic organisms remains undetermined.

2.3.3.4 Parabens

Parabens (alkyl-p-hydroxybenzoates) are antimicrobial preservatives found in cosmetics, toiletries, pharmaceuticals, and certain types of food (Daughton & Ternes, 1999). Seven distinct types of parabens are currently in use, namely, benzyl, butyl, ethyl, isobutyl, isopropyl, methyl, and propyl. The number of parabens is expected to grow in the future. Methyl- and propylparaben are the most commonly used in cosmetics and are usually co-applied to increase preservative effects (Peck, 2006).

Only a limited number studies have inspected paraben concentrations in WWTP and surface water (Brausch & Rand, 2011).

Methyl- and ethylparaben seem to be least acutely toxic of the parabens with LC_{50} values approximately three times greater than benzylparaben (Terasaki et al., 2009). Benzylparaben appears to be most acutely toxic (Terasaki et al., 2009). It has been reported that increasing chain length of parabens' substituents can increase paraben acute toxicity to bacteria. This appears to be true for other trophic groups as well (Brausch & Rand, 2011).

The only data on the chronic effects of parabens to aquatic organisms comes from a single known study examining toxicity in *D. magna* and *Pimephales promelas* (fathead minnow) (Dobbins et al., 2009). This study found that benzyl- and butylparaben had the highest toxicity to invertebrates and fish whereas methyl- and ethylparaben appeared to have the lowest toxicity. This correlates well with the findings of acute toxicity studies and previous studies that indicated increased chain length of parabens increases toxicity. It must be noted that chlorination also considerably enhances the toxicity of parabens to both bacteria and *D. magna* (Terasaki et al., 2009).

Benzyl-, butyl- and propylparaben might possibly cause adverse effects to aquatic organisms based on available environmental concentration and toxicity data. Dobbins et al. (2009) reported that parabens incur a limited hazard to aquatic organisms. Dobbins et al. (2009) did, however, mention that benzyl-, butyl- and propylparaben can stimulate low-level oestrogenic responses in organisms that are chronically exposed to parabens. Analysis of environmental concentrations suggests that the risk to aquatic organisms is only minimal. This is because effect concentrations tend to be a thousand times higher than observed concentrations within surface waters (Brausch & Rand, 2011).

2.3.3.5 UV filters

Recent understanding of the various hazards that are posed by UV radiation to humans has caused an amplification in the usage of UV filters (Brausch & Rand, 2011). UV filters are found in sunscreen products and cosmetics to provide fortification against UV radiation. These filters can either be organic (absorb UV radiation, e.g. methylbenzylidene camphor) or inorganic micro-pigments (reflect UV radiation, e.g. ZnO, TiO_2) (Brausch & Rand, 2011). For the purpose of this project, focus will be given to organic (absorbent) products.

UV filters tend to enter the environment directly or indirectly. This can be directly from being washed off the body while swimming or during other recreational activities, or it can enter the environment indirectly via WWTP effluent (Brausch & Rand, 2011). While UV filters are used at high levels and are thus likely to enter aquatic environments, there is a lack of data on actual occurrence information largely due to a lack of suitable analytical methods (Brausch & Rand, 2011).

A study indicated that UV filters do not appear to be acutely toxic to aquatic organisms (Fent et al., 2010). UV filters tend to bioaccumulate and recent studies have also shown that they also have the potential for oestrogenic activity. These studies used fish (*P. promelas* and *O. mykiss*) to indicate that several UV filters can cause oestrogenic effects and may also adversely affect fecundity and reproduction (Brausch & Rand, 2011).

Even though PCPs can be released at levels exceeding other compounds, including pharmaceuticals, there is a lack of research on the identification of environmental concentrations and potential toxicity (Brausch & Rand, 2011). PCPs in the environment are also repeatedly replenished through normal usage and are thus persistent or at the very least pseudo-persistent compounds that warrant acute and chronic studies (Brausch & Rand, 2011).

Studies into both acute and chronic toxicity are also needed to fully elucidate the potential effects and risks that the release of PCPs into surface waters entails. As is the case with pharmaceuticals, studies examining the effects of PCPs on benthic invertebrates are severely lacking (Brausch & Rand, 2011).

The potential for PCPs to bioaccumulate and their propensity to cause oestrogenic and endocrine effects need to be studied and investigated as these are the areas of greatest concern as most completed studies reported little short- or long-term toxicity (Brausch & Rand, 2011).

2.3.4 Contrast media

Most iodinated X-ray contrast media (ICM) are derivatives of 2,4,6-triiodobenzoic acid with polar carboxyl and hydroxyl moieties in their chains. Some have one or several free carboxyl groups, while others are neutral compounds as they are amide derivatives (Pérez & Barceló, 2007b). They all have the shared characteristic of featuring iodine atoms in the molecule, as these are responsible for absorption of X-rays, which is what makes these compounds suitable for use during X-ray diagnostic processes (Pérez & Barceló, 2007b).

The most commonly used pharmaceutical (approximately 3.5×10^6 kg per year) administered intravenously is ICM (Pérez & Barceló, 2007b). ICMs are perfectly suited for use during diagnostic tests (the imaging of organs or blood vessels) due to their stable metabolic characteristics while inside the body, which allows for rapid elimination via urine or faeces after use (Pérez & Barceló, 2007b). It should be noted that belated side effects can still occur at frequencies of 1–3% in patients who have been exposed to ICMs. These side effects are allergic/allergy-like adverse effects that includes the *de novo* synthesis of cysteinyl leukotrienes (Pérez & Barceló, 2007b). The negative effects associated with ICM were discovered in hospital waste water where these compounds contributed considerably to organically bound halogens that could be absorbed onto activated carbon (Kümmerer et al., 1998).

2.3.5 Flame retardants

Flame retardants are anthropogenic environmental contaminants that are used at relatively high concentrations for numerous purposes (Segev et al., 2009). Brominated flame retardants (BFRs) (the most common and popular flame retardants currently available on the market) are considered toxic, persistent and bioaccumulative (Segev et al., 2009). Flame retardants are used at reasonably high concentrations (5–30%) in numerous indoor and outdoor applications, which include the manufacture of electronic equipment, textiles, plastic polymers, televisions, computers, microwave ovens, copy machines, lamp shades, textiles, furniture and in the car industry (Alaee et al., 2003). Globally the demand for BFRs continues to rise. It is estimated that more than 200 000 tonnes of BFRs are produced in the USA alone each year, and that more than 1.5 million tonnes are produced globally each year (Alaee et al., 2003). The primary use of flame retardants are to protect materials against ignition and to minimise fire-related damage (Segev et al., 2009).

As with most halogenated organic compounds, BFRs tend to have restricted biodegradability, thus they are persistent and tend to accumulate in the environment (Segev et al., 2009). It is worth mentioning that the toxicity of the compounds towards fauna, flora and humans can be naturally altered to be less or even more toxic than the original compound (Segev et al., 2009). Flame retardants are introduced into the environment in a multitude of ways. They can, for example, be present in waste waters from industrial facilities that manufacture flame retardants (including facilities that integrate these compounds into the products they produce) (Birnbaum & Staskal, 2004). The volatilization and leaching from products during manufacture or usage and the breakdown of foam products are also ways that flame retardants can enter the environment (Segev et al., 2009). In addition, the disposal of products (e.g. electronic equipment), leaching from landfills, burning and recycling of waste, or adsorption onto dust particles can also result in flame retardants ending up in the environment (Birnbaum & Staskal, 2004).

Additive flame retardants tend to be released into the environment at a greater rate than reactive flame retardants (Alaee et al., 2003). When a flame retardants enters the environment, attachment to particles can lead to these compounds being able to travel great distances (recent studies have even found levels of BFRs in the Arctic) if they travel with airborne dust particles or via water bodies such as rivers

(Segev et al., 2009). During transport in the water body, these molecules can adhere to solid particles and can thus become part of the suspension load and later the deposition load (Segev et al., 2009). After being deposited, these compounds will form part of the sediment of the region. Therefore, traces of halogenated and organophosphorous-containing flame retardants can be found in terrestrial, freshwater and marine environments, often at great distances from the site of their production/release (Birnbaum & Staskal, 2004).

Increased lipid solubility and reduced water solubility are directly associated with the halogen moiety of organic compounds. Additionally, the toxicity of compounds can be increased by the halogen substituent and its potential organohalide metabolites (Birnbaum & Staskal, 2004). Thus, many BFRs are toxic (acute and chronic), persistent and bioaccumulative in the environment, due to bromide substituents (Birnbaum & Staskal, 2004). BFRs have been found in plants and animals (including humans) throughout the food chain (Segev et al., 2009).

Human tissue, blood serum and breast milk of exposed individuals (e.g. people working in the production of BFRs) have all been known to contain BFRs (Sjödin et al., 2003). BFRs have numerous hazardous qualities such as immunotoxicity, cytotoxicity, neurotoxicity, endocrine-disrupting capabilities, genotoxicity, mutagenicity, carcinogenicity and teratogenicity (Birnbaum & Staskal, 2004). Despite their potentially devastating attributes, limited data is available on BFRs, particularly regarding the effects of BFRs on the environment (including environmental fates and biodegradability potential), plants, animals and humans (Segev et al., 2009).

2.3.6 Phthalates

Phthalate esters or phthalates are the dialkyl or alkyl aryl esters of 1,2-benzenedicarboxylic acid (phthalic acid). The name phthalate is derived from phthalic acid, which refers to three isomers, namely, ortho-isomer or phthalic acid, para-isomer or terephthalic acid, and meta-isomer isophthalic acid (Liang et al., 2008). Phthalates can be found in multiple environments including air (Wensing et al., 2005), soils, sediments, landfill leachate (Schwarzbauer et al., 2002), and natural waters (Stales et al., 1997). The diversity of the environments within which phthalates can be found is attributed to the popularity and widespread use of plastic. Phthalates are not chemically bonded to the plastic polymer when used as plastizers, thus rendering them able to transfer from the plastics into the environment (after a sufficient time period).

Phthalates are produced in massive quantities during the manufacturing of various plastics. As a result of their release during these processes, including use and disposal, phthalates have become widely distributed throughout the environment (Liang et al., 2008). This is cause for concern as phthalates and their metabolites are hepatotoxic, teratogenic, and carcinogenic by nature, thus they have the potential to cause serious damage to the environment and humans (Matsumoto et al., 2008).

2.3.7 Polycyclic aromatic hydrocarbons

PAHs comprise carbon and hydrogen atoms arranged in the form of fused benzene rings (linear, cluster or angular arrangement) (Maliszewska-Kordybach, 1999). They feature strong mutagenic, carcinogenic and toxic properties. PAHs consist of thousands of compounds in the environment and 16 of these are defined as Environmental Protection Agency (EPA) priority PAH compounds. Individual PAHs can feature substantial differences in their physical and chemical properties (Maliszewska-Kordybach, 1999). Generally, PAHs with a lower molecular mass are more water soluble, more volatile and less lipophilic than higher molecular mass PAHs (Van Jaarsveld et al., 1997).

Some PAHs are resistant to environmental degradation and can thus remain in the environment for long periods of time, which gives them ample opportunity to cause environmental impacts. The dispersion of these chemicals are also a concern as some of them are semi-volatile, meaning that under normal environmental conditions they are in constant motion between the surface and atmosphere of the earth in repeated, temperature-driven cycles of deposition and volatilization (Maliszewska-Kordybach, 1999).

PAHs are produced and released during all processes of incomplete combustion of organic materials and as such they occur over a wide area. The size of this area and the concentrations of PAHs in the environment were greatly expanded during the last century of industrial development (Wild & Jones, 1995). Before being deposited via atmospheric precipitation on soils, vegetation or sea and inland waters, PAHs can travel great distances through the air (Wild & Jones, 1995). The presence of PAHs in all the various compartments of the environment may pose a threat to all living organisms (Maliszewska-Kordybach, 1999).

Anthropogenic PAHs can be separated into two categories based upon their origin: the combustion of materials for energy supply and the combustion for waste removal (Wild & Jones, 1995). The first category includes both stationary and mobile sources. Examples of mobile sources include industries (mainly coke and carbon production, petroleum processing, aluminium sintering, etc.), residential heating (furnaces, fireplaces, stoves, and gas and oil burners), power and heat generation (coal, oil, wood and peat power plants). Mobile sources include examples that are mainly part of the transport sector like cars, trains, airplanes and ships (Maliszewska-Kordybach, 1999). Means of transport can affect a country's estimated annual PAH emission quantities as there is a difference between gasoline and diesel engines (Maliszewska-Kordybach, 1999). The second category covers the incineration of both municipal and industrial wastes (Maliszewska-Kordybach, 1999). Miscellaneous sources of PAHs include unregulated fires (which includes the burning of biomass) such as agricultural burning, recreational fires and crematoria (Wild & Jones, 1995).

2.3.8 Disinfection by-products

Disinfectant by-products (DBPs) are formed by the interaction between the chemicals used as a part of water sanitation and biotic as well as abiotic matter (Diamond et al., 2011). Strong oxidising agents such as chlorine and chloramine are often used as disinfection agents in water for pathogen removal, odour elimination and to form a lingering disinfectant presence to ensure the safe transport of water to consumers. It should be noted, however, that these strong oxidising agents can react with fulvic and humic acids, amino acids, and other organic tissues once they have been ingested (Diamond et al., 2011).

The negative health effects associated with DBPs can be quite severe (Nieuwenhuijsen et al., 2000). The risk this class of ECP cannot be ignored as it has been linked to numerous problems in pregnant women specifically. These problems include low birth weight, preterm delivery, spontaneous abortions, stillbirth and birth defects, major cardiac defects, oral cleft, and respiratory, and neural tube defects (Nieuwenhuijsen et al., 2000).

Nieuwenhuijsen et al. (2000) recommended that large methodically designed epidemiological studies be carried out to determine if the risks that have been attributed to DBPs, especially in the case of pregnant women, are factual and correct. The authors did, however, note that these studies could prove costly, but that these costs can be minimised through proper planning (Nieuwenhuijsen et al., 2000).

2.4 Sources and Environmental Pathways of Emerging Chemical Pollutants

Certain organic chemicals have been proven to be harmful to fish, other animals and potentially also humans (Diamond et al., 2011). Therefore, there has been a great effort to study the fate, occurrence and ecotoxicology of ECPs in the aquatic environment. Sources and environmental pathways of ECPs have been increasingly associated with waste and waste waters arising from industrial, agricultural and municipal activities (Jorgensen & Halling-Sorensen, 2000). Residues of these biologically active compounds can enter the environment via different transport pathways such as emissions during manufacture, disposal of products and unused or expired medicines, human and animal excretion in urine and faeces, direct discharge of aquaculture products, and manure and slurry spreading (Jorgensen & Halling-Sorensen, 2000) (Figure 1).

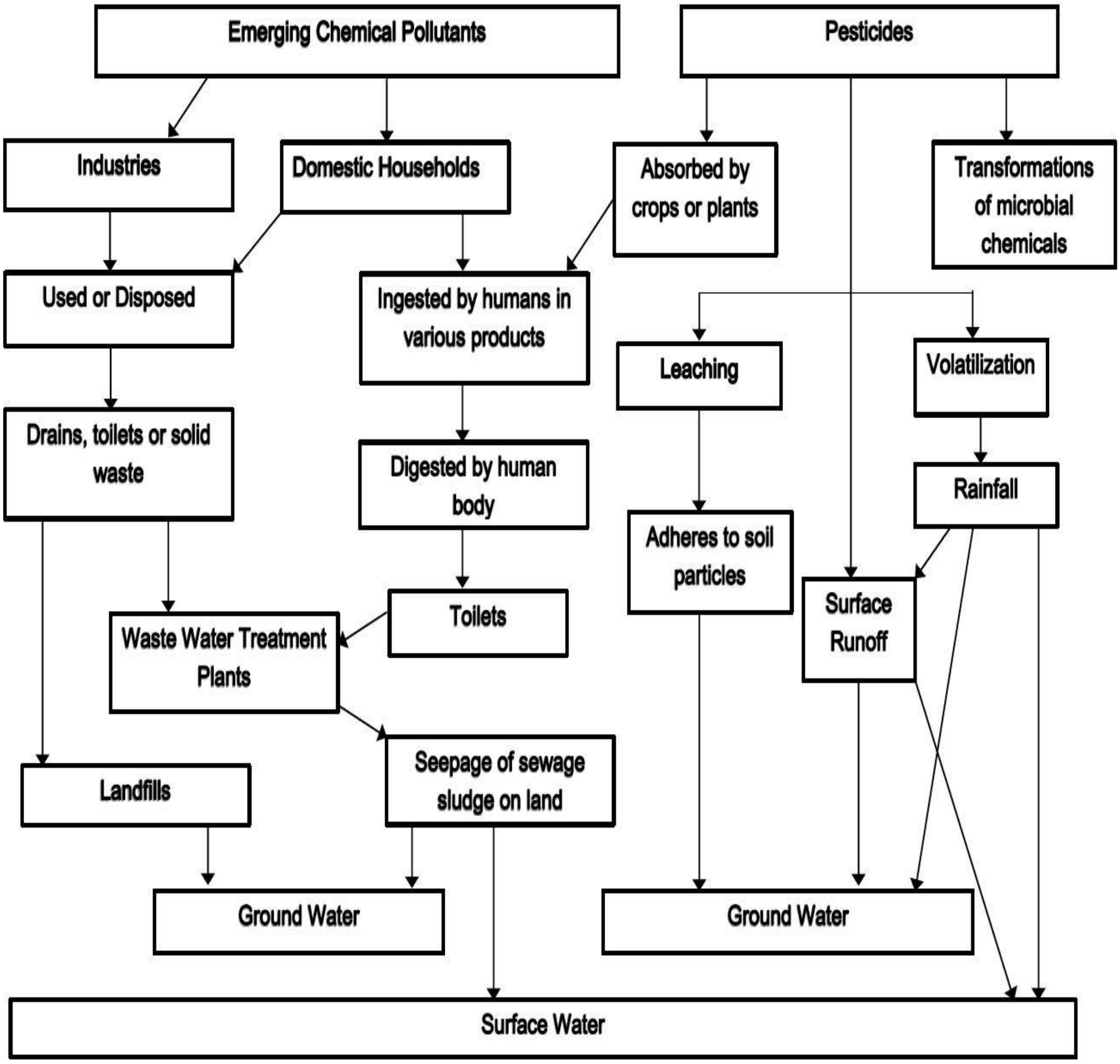

Figure 1: Sources and environmental pathways of ECPs to surface waters (adapted from numerous literature sources)

Veterinary drugs (used to treat and prevent diseases in farming) and their metabolites are prone to contaminate soil and groundwater, but they are intentionally introduced into the environment when liquid manure is sprayed on agricultural fields. The transport of veterinary drugs to groundwater can be through leaching or run-off from livestock slurries, while sorption of the drug onto soil particles can delay its distribution. Human pharmaceuticals enter aquatic systems after ingestion and subsequent excretion in the form of the non-metabolised parent compounds or as metabolites through WWTPs (Pérez & Barceló, 2007a). If the pharmaceuticals and their human or animal metabolites pass through WWTPs, they can then enter rivers or streams. Alternatively, they can undergo leaching and enter the groundwater. In addition, run-off from fields treated with digested sludge can cause pharmaceuticals to reach surface waters.

2.5 Movement and Degradation of Emerging Chemical Pollutants in the Environment

Once ECPs enter a water resource, they can follow multiple pathways that may alter their concentration. Certain of these pathways involve the partitioning of these chemicals, which mean that they remain intact but can escape into the gas phase or be adsorbed and become part of the sediment of the water body (García et al., 2011). Degradation can also occur in some of the possible pathways. These chemicals can be degraded by light (photolysis) or they can be ingested and broken down by organisms (metabolisation) (Gavrilescu et al., 2014).

The degree to which partitioning and degradation takes place depends on numerous physical factors including: retention time in various media, sediment properties, pH, water temperature, light penetration, and degree of eutrophication (Haarhoff et al., 2015). If the exact details of partitioning and degradation rates are required for a study, a site-specific investigation is required that considers the uniqueness of the aforementioned physical factors of the specific site.

2.5.1 Bioaccumulative potential, ingestion and metabolisation of ECPs by organisms

ECPs are readily absorbed by organisms through their skin, respiratory and gastrointestinal tracts, before being distributed throughout the tissues of the organism (Gavrilescu et al., 2014). The bioaccumulation potential of ECPs tends to be relatively low, thus there generally tends to be low concentrations of these chemicals found in tissue (Haarhoff et al., 2015). It is worth noting that ECPs are often more concentrated in specific areas and organisms, as well as tissues within a specific organism than in others. The liver and kidneys of organisms can have high levels of ECPs within them whereas the body fat, brain, and muscle tissue usually have lower levels of ECPs (Haarhoff et al., 2015). There are exceptions to this as certain ECPs accumulate in the body fat as they are lipophilic.

The potential for a substance to accumulate or to be retained within the body is also an important consideration when assessing the risk that it poses. The properties of these substances are such that the body cannot readily remove them, so they steadily build up with successive exposures. The threat they pose to the organism can thus be sustained for long periods of time (Joint Research Centre, 2003).

Lipophilic substances are an example of substances that have the potential to accumulate within the body (Joint Research Centre, 2003). While there is no direct correlation between the lipophilicity of a substance and its biological half-life, substances with high log P (partition coefficient) values tend to have longer half-lives. Thus, there is the potential for highly lipophilic substances ($\log P > 4$) to accumulate in individuals if they are frequently exposed to that substance (Joint Research Centre, 2003). The concentration of the substance within the body declines at a rate determined by the half-life of the substance after exposure has ceased. Other substances that can accumulate within the body include poorly soluble particulates that deposit in the alveolar region of the lungs, substances that bind irreversibly to endogenous proteins and certain metals and ions that interact with the crystal matrix of bone (Joint Research Centre, 2003).

Highly hydrophobic substances can accumulate in sediments to concentrations at which they might exert significant toxic effects (Joint Research Centre, 2003; Gavrilescu et al., 2014). This is of great concern in the marine environment where the sediment may act as a permanent sink for highly hydrophobic substances that can be accumulated to a large extent. As marine sediment constitutes an important compartment of marine ecosystems, the accumulation of potentially harmful substances can have far-reaching negative effects for numerous marine ecosystems (Farré, 2008).

2.5.2 Volatilization of ECPs

The partitioning of an organic chemical between water and air is a physical property described by Henry's Law Constant, H (Joint Research Centre, 2003). The magnitude of H provides an indication of which of the two phases, water and air, a chemical will tend to partition into at equilibrium. Chemicals with low values of *H* will tend to partition into the aqueous phase (Joint Research Centre, 2003). Since air and water are the major compartments of most model ecosystems, and water is considered to act as the link between all of the compartments, knowledge of the value of *H* is very important when assessing the environmental risks associated with a chemical. The Henry's Law Constant is expressed either as the ratio of the partial pressure in the vapour phase and the concentration in water [*H* ($Pa \cdot m^3 \cdot mol^{-1}$)], or as the ratio of the concentrations in air and water (*H'*, dimensionless) (Joint Research Centre, 2003).

The release of organic ECPs from the water phase to the atmosphere is dictated by its vapour pressure, which shares a direct relationship with temperature (Haarhoff et al., 2015). Thus, at higher temperatures (elevated water temperature), an ECP volatilizes at an increased rate. Once the ECP enters the atmosphere, it can either remain as a vapour or it can be adsorbed by suspended atmospheric particles.

2.5.3 Photolysis as a process of degrading ECPs

Sunlight can break some of the chemical bonds of an ECP when the ECP is directly exposed to sunlight (Haarhoff et al., 2015). This mechanism of degradation is of specific importance in two scenarios; the first being once an ECP has volatilized and enters the atmosphere. The second scenario occurs in instances where sunlight penetrates a water body. South Africa receives significant solar radiation and as such photolysis can play a larger role in our ecosystems than in areas that receive less sunlight such as Europe, Canada and large parts of the USA (Haarhoff et al., 2015). The precise effect of photolysis on ECPs in South Africa is yet to be studied and accurately quantified.

2.5.4 Adsorption of ECPs into sediments

Sediments have been identified as a major carrier of ECPs in water. The octanol-water partition coefficient of a substance describes the partitioning of the compound between the water and the surrounding sediment. The log *n*-octanol-water partition coefficient (log K_{ow}) is a measure of the hydrophobicity of a chemical. As such, log K_{ow} is a crucial parameter when assessing environmental fate (Joint Research Centre, 2003). Many distribution processes are driven by log K_{ow}, for example, sorption to soil and sediment, partitioning into air and bioconcentration. ECPs are commonly highly hydrophobic and very insoluble in water and thus they tend to be adsorbed onto finely dispersed colloids and particulates (Amdany et al., 2014).The free dissolved concentrations of ECPs in water are therefore frequently several orders of magnitude lower than their total concentrations. These free dissolved concentrations are generally within the $ng \cdot L^{-1}$ to $pg \cdot L^{-1}$ range, which is usually too low for accurate quantitative chemical analysis by traditional methods (Amdany et al., 2014). This means that the reliable and accurate analysis of free dissolved ECPs in natural water can be challenging and difficult. Therefore, it is quite common for many sampling problems to occur (Amdany et al., 2014).

The sorption to soil and sediment components is a determining factor for the mobility of chemicals (Gavrilescu et al., 2014). This property accounts for the distribution among soil, sediment and water phases, and for volatilization from soil surfaces, and influences the chemicals bioavailability and hence its transformation by soil microbes, for example. The extent of sorption to soil and sediment is governed by a variety of physicochemical properties of both the soil and the contaminant. The heterogeneous soil chemistry and physics due to the variant proportions of the major components – mineral and organic matter, water, air and (micro)organisms – account for the differences in the binding capacity of different soils (Joint Research Centre, 2003). The relevant parameters include organic carbon content, clay content, humidity, pH-value, cation exchange capacity, and temperature (Joint Research Centre, 2003). The underlying processes of sorption may be due to Van der Waals interactions, hydrophobic interactions, hydrogen bonding, charge transfer interactions, ligand exchange and ion bonding, direct and induced ion-dipole and dipole-dipole interactions and covalent bonding (Joint Research Centre, 2003). The sorption of non-polar substances, generally to the organic matter of the soil or sediment can be regarded as a distribution process between the polar phase of the soil water and the organic phase of the soil components (Joint Research Centre, 2003). The equilibrium constant of this partitioning between solid and solution phases constitutes the adsorption coefficient for soil and sediments (Joint Research Centre, 2003).

2.6 Efficiency of Emerging Chemical Pollutant Removal by WWTPs

The occurrence of ECPs in waste water effluent has been studied and it has been found that some of these chemicals are removed effectively by the waste water treatment process, although this process was not developed or intended to remove these chemicals (Haarhoff et al., 2015). There are, however, certain ECPs that are not removed (or only partially removed) by traditional waste water treatment processes (Haarhoff et al., 2015). There is currently a lack of knowledge on precisely what the impact of each process within the WWTP is on the removal of ECPs from the effluent. The presence of ECPs in water used for human consumption is of great concern due to the multiple potential chronic effects these chemicals can have on human health (Haarhoff et al., 2015).

An example of ECPs found in waste water effluent can be seen in a study done in South Africa that examined the presence of ECPs in waste water effluent and found a multitude of chemicals present including pharmaceutical products used to treat hypertension, ARVs, analgesics, antibiotics, and hormones from natural and contraceptive sources (Osunmakinde et al., 2013). These ECPs then flow with the water and unavoidably end up being used or consumed by humans. This idea is further strengthened by another study done in 2013 (Patterton, 2013) that found certain ECPs to be present in South African drinking water.

Dilution is a vital consideration when assessing the risk and potential impact that can be caused by ECPs (Deblonde et al., 2011). Differing volumes of waste water that enter a WWTP may lead to varying concentrations of ECPs. The volume of waste water that passes through a WWTP in conjunction with the number of households and businesses that are connected to the sewage system are thus key considerations in determining the likelihood of detecting ECPs in WWTPs (Deblonde et al., 2011). It also influences the chances that these ECPs will have adverse effects on the surrounding environment (Deblonde et al., 2011). The source of waste water is vital information required when sampling for specific pollutants (Deblonde et al., 2011).

Fluctuations in temperature, precipitation rate and solar radiation have been found to influence the number of molecules found suspended in waste water (Deblonde et al., 2011). The precise process behind the elimination of pharmaceuticals is largely unknown; however, the processes of biodegradation and sorption are known to contribute to the process as a whole (Deblonde et al., 2011). It is worth noting that temperature influences both of these processes. Another elimination process of pharmaceuticals from waste water is photodegradation. This process relies on sunlight and as such it is less prominent during the winter months when solar radiation is less intense (Deblonde et al., 2011). It is then very interesting to note that it has been reported that the elimination rate of ibuprofen was reasonably constant even with seasonal weather changes (Deblonde et al., 2011).

A two-year long analysis of WWTPs (Bueno et al., 2012) yielded valuable information regarding the various treatment processes for waste water in WWTPs, specifically surrounding their efficacy of removing ECPs. It was found that activated sludge biological treatment was the most efficient at removing stimulant compounds (> 80%), UV filters (> 86%) and some synthetic fragrances, analgesics/ anti-inflammatories or disinfectants (> 70%) (Bueno et al., 2012). Blood lipid lowering agents, diuretics and beta blockers featured mean removal efficiencies of 50%. It should, however, be noted that certain ECPs, most notably some antibiotics and carbamazepine, featured very poor or no elimination at all (Bueno et al., 2012). This persisted even after the waste water was put through secondary treatment. This means that effluent from WWTPs can still contain ECPs and that these ECPs are thus continuously being discharged into water resources (Bueno et al., 2012).

These results confirmed that ECPs can remain present in water even after it has been treated by WWTPs. This can be due to two predominant reasons. The first being that certain ECPs are resistant to removal and that the current waste water treatment processes are insufficient at removing them effectively (Bueno et al., 2012). The second reason that ECPs can remain in water after it has been treated is because the chemicals are being discharged into waste water continuously and in large quantities (Bueno et al., 2012). This means that even if waste water treatment processes can remove the chemical, it simply occurs in such large quantities that it remains a threat or potential problem even after the water has been treated. These two reasons explain why effluent from WWTPs is an important pathway for ECPs to be discharged and thus to potentially contaminate a country's water resources.

Both domestic and industrial waste chemicals that are disposed of via water will typically go through at least one WWTP before they end up in either surface or groundwater (Haarhoff et al., 2015). Mining and agricultural waste has an increased potential to end up in surface and groundwater due to discharges directly into waterways (Haarhoff et al., 2015).

There is currently a lack of data on the efficiency of ECP removal from water by WWTPs in South Africa (Haarhoff et al., 2015). Most South African WWTPs are conventional plants, which means that the processes they follow are coagulation, flocculation, phase separation by settling/flotation, rapid filtration and lastly chlorination (Haarhoff et al., 2015). More modern and sophisticated processes are starting to come into general use, and as such they will also be briefly discussed.

Pharmaceutical residues, antibiotics, steroid hormones and fragrances have been reported to be the most commonly occurring trace organic compounds in secondary and tertiary treatment municipal effluents as well as water bodies receiving these waste water discharges (Crook, 2010). These chemicals are thus of specific importance in a South African context as South Africa mostly uses traditional waste water treatment methods.

2.6.1 Coagulation and flocculation

Coagulation and flocculation are generally ineffective at removing ECPs and specifically EDCs from drinking water (Snyder et al., 2009). There are a limited number of substances that can be effectively removed by coagulation and flocculation; however, the removal efficiency is generally below 20% (Haarhoff et al., 2015).

2.6.2 Disinfection by means of oxidation

The most common oxidant in South Africa is chlorine, which is used for disinfection in WWTPs (Haarhoff et al., 2015). Other oxidants less commonly used by WWTPs in South Africa include ozone, chlorine dioxide and UV irradiation. Chlorine has been found to be very effective at removing ECPs and specifically EDCs from drinking water as it removes 95% of these substances (Haarhoff et al., 2015). Regardless of being sufficient for controlling several target compounds, it does have a few limitations. Chlorine dioxide is a stronger oxidant than chlorine and it removes considerable proportions of many compounds (Haarhoff et al., 2015). It should, however, be mentioned that certain ECPs such as caffeine and ketoprofen are recalcitrant to chlorine dioxide oxidation. Chlorine and chlorine dioxide target and react mainly with functional groups such as amines and phenols. However, concerns have been raised regarding potential adverse health effects (including reproductive effects) of the disinfection by-products of chlorination of water (Nieuwenhuijsen et al., 2000).

Ozone, the strongest oxidant, is very effective at removing and transforming ECPs and EDCs in water resources. Many of these substances can be oxidised by up to 90–99% by ozone, thus ozone is considered extremely efficient at eliminating these substances (Haarhoff et al., 2015).

The efficiency of UV radiation at removing ECPs and EDCs is still a contested and debated subject. UV radiation, at the radiation doses normally used for the treatment of drinking water, is considered to be ineffective at removing these substances according to a review sponsored by the EPA (Snyder et al., 2009). Other scientists have contrasting views and believe that medium-pressure UV lamps (which are widespread at WWTPS internationally and are also used at a limited number of South African plants) are very proficient at eliminating certain ECPs by direct photolysis (Sharpless & Linden, 2003).

2.6.3 Advanced oxidation

Advanced oxidation processes consist of a combination of UV, hydrogen peroxide and ozone treatments (UV/hydrogen peroxide; ozone/hydrogen peroxide; and UV/ozone) (Haarhoff et al., 2015). These combinations create hydroxyl radicals that bind to, react with and transform ECPs and EDCs non-selectively. This can lead to a drastic increase in removal efficiency, for example, the removal efficiency of ibuprofen is doubled (Haarhoff et al., 2015). Advanced oxidation processes are consequently considered to be very effective processes for oxidising and removing ECPs and EDCs from drinking water; however, they are only slightly more effective than ozone alone.

2.6.4 Activated carbon adsorption

Activated carbon adsorption has been found to be effective at removing ECPs and EDCs from drinking water when used in both powdered and granular form (Delgado et al., 2012) due to their hydrophobic properties. Activated carbon can however not successfully remove all polar compounds (Delgado et al., 2012).

2.6.5 Membrane filtration

There are a wide variety of membrane types that are used during membrane filtration. Together, they form a continuum. Membrane types include microfiltration (the coarsest pore size), ultrafiltration, nanofiltration to the smallest pores in reverse osmosis (Haarhoff et al., 2015). Apart from the sizes of the membrane pores, there is also a distinction between high-pressure membranes and low-pressure membranes.

Low-pressure membranes typically have larger pore sizes than the size of most of the molecules of both ECPs and EDCs. Consequently, these low-pressure membranes are typically ineffective at removing ECPs and EDCs from drinking water (Haarhoff et al., 2015). It should be noted that some elimination of these pollutants will occur, but that is largely due to the direct absorption that occurs on the surface of the membrane. High-pressure membranes, due to their smaller pore sizes, are much more successful in removing ECPs and EDCs. These membranes have been commonly reported to be more than 90% effective at removing ECPs and EDCs from drinking water (Haarhoff et al., 2015).

2.7 Emerging Chemical Pollutants and Legislation

Water quality legislation aims to protect, govern and manage water resources to meet the needs of the current generation while not compromising the ability of future generations to meet their own needs pertaining to water resources. Acceptable limits determined through scientific inquiry are often communicated through these laws, which makes the relationship and communication channels that exist between scientists and legislators’ imperative for the effective protection of water resources.

How a river is used within South Africa largely dictates the level of pollution that is acceptable, thus leading to a scenario where different rivers can have different quality standards. For example, a river flowing through a densely populated area is typically more polluted than a river flowing through an area with a lower population number. Studies on the hazards and risks that are posed by ECPs towards both human and ecological health are still relatively novel; therefore, more information is required regarding these negative impacts. There is also a need to find empirical and undeniable evidence for the impacts they can cause. Lack of empirical evidence creates a scenario where legislation is lacking surrounding ECPs and what the exact acceptable limits of ECPs in water resources should be. EDCs can be seen as an exception to this statement as some legislation surrounding EDCs does exist in some parts of the world; however, if this is sufficient and how effectively it is being implemented remains questionable. EDC legislation has also not been universally ratified and many countries (including South Africa) lack legislation in this regard.

2.7.1 South African legislation

The management of South African water resources is largely governed by the South African National Water Act published in 1998 (DWA, 1998). The restructuring of past discriminatory laws surrounding water resources forms the core of this piece of litigation, with a focus on equal ownership and right to South Africa's water resources by its citizens (DWA, 1998). The scarce and valuable nature of water in South Africa and thus the import of sustainable resource management was also a central consideration when the National Water Act was proposed and later written and ratified (DWA, 1998).

Circumventing the overexploitation of water resources and ensuring the effective sustainable social and economic development of these resources are governed by the National Water Act (DWA, 1998). Equity, sustainability and efficiency form three central pillars around which the National Water Act is based (DWA, 1998). Universal access by the citizens of the country to the water resources of South Africa and to the benefits that can be attained from these water resources is the formative idea behind the equity pillar. The conservation of water resources for future generations, while still encouraging and facilitating the utilisation of water resources for economic and social development for the benefit of the current generation, forms the core philosophy of the sustainability pillar. Reducing water wastage, as well as promoting the most efficient and equitable use of water for economic and social development, constitutes the efficiency pillar.

Chapter 2 of the National Water Act describes the National Water Resource Strategy, which is of importance to all water users and institutions as it contains laws pertaining to them (DWA, 1998). The Act expedites the proper management of South Africa's water resources by establishing the guidelines surrounding the utilisation, management and development of the water resources within South Africa (DWA, 1998). These guidelines include measures on water resource management at both a catchment and regional level. There is provision for an obligatory five-yearly update of the National Water Resource Strategy. This may provide an effective mechanism for including ECPs and especially EDCs in South African water quality legislation, as these substances could potentially lead to hazardous conditions for the people of the country.

The level of conservation that a specific water resource requires is based on analysing and specifying definitive limits surrounding water quality and quantity. Quantity of water requires looking at how much water is available; how much is required and is extracted from the specific water resource. The physical, chemical and biological aspects of a specific water body determine the quality of the water found within it. The health of various aquatic ecosystem components including the flora contained within and surrounding the water body, insects, birds and mammals must also be analysed to determine water quality.

Preventing pollution is a pivotal part of the conservation and management of water resources. The National Water Act supplies legislation surrounding pollution prevention in its third chapter (DWA, 1998). This chapter also assigns culpability to water pollution by stating that the individual who caused the pollution is responsible for mediating it and that landowners are responsible for preventing the pollution of water bodies whenever possible. Failure of the responsible person to do so can mean that the relevant catchment management agency may take prevention or mitigation steps, and that the entity that caused the pollution will be responsible for paying for the cost of both mitigation and prevention. This creates a problematic scenario surrounding ECPs that lack litigation, as there is no specified legal requirement about the removal of ECPs and EDCs from water resources. This means that nobody will be held legally accountable for the damage these substances can cause; therefore, ECPs and EDCs will largely remain in the water resources as it is unlikely that additional effort or expense will be made for the sake of removing these substances from the water resource.

In order to find a balance between the need to use water bodies for the purpose of economic and social development and the need to conserve water bodies, the Water Resource Classification System (WRCS) was implemented. This system classifies water bodies on the grounds of the characteristics of the water bodies in term of their purpose in aiding the social and economic development of South Africa (requires lower levels of protection) as well as their ecological importance (requires higher levels of protection). Sustainability in terms of both development and conservation is key to the WRCS. The classification process involves numerous compromises between the goals of development and conservation and forms an imperative part of the overarching goal of the protection of water bodies. Compromises can also be at a smaller scale such as where during a river's flow water will be used (for development) and where it will be conserved. Using water upstream can reduce both the quality and quantity of the water that will be available for downstream use. The inverse of this scenario where water is only used downstream and not upstream can lead to a lack of socio-economic development occurring in the upstream areas as well as dissatisfied people.

The WRCS categorises water resources into various classes. It then assigns a specific management goal for each individual water body by taking its unique characteristics into account. These characteristics include the existing condition of the water resource and then surmising the developmental and ecological processes that are reliant on it. A cost benefit analysis is then conducted to analyse the available options of development and conservation of the relevant water body while considering the surrounding environmental and socio-economic conditions. The WRCS groups water resources into three classes ranging from water bodies that are heavily developed and used for socio-economic development to water bodies that feature little or no development or exploitation for socio-economic purposes. A determination of the quantity and quality of water required for both optimal ecosystem functionality and socio-economic development will be included in the ensuing conservation process as well as the classification of the individual water resource.

Initiation of the WRCS involves defining the existing condition of the water resource from an environmental point of view. After the existing condition has been accurately determined, a planning phase is implemented that is concerned with what the ideal envisaged future condition of the water resource should be. Consultation with relevant stakeholders forms an imperative part of this process. The ideal envisaged future condition of the water resource relies upon assigning preferred attributes that are epitomised within a management class. This management class is monitored and managed according to a pre-approved set of traits determined by the Department of Water Affairs and the general public.

After the water resource classification has been concluded, it will inform the decision of the Minister of Water Affairs surrounding the management class, the various reserves surrounding the relevant water resource, as well as the quality requirements of the resources. Management classes will include a description of the existing condition of the water resource and will use it to describe to what extent the water resource can be used, exploited or developed. Information surrounding the acceptable limits of the quantity, circulation and quality of the reserves surrounding the specific water resource and thus the percentage of the water resource that can be used will be included in the management class. The management class of a water resource can thus have substantial socio-economic and environmental consequences.

It should be noted that very few organic compounds have been included in the drinking and surface water guidelines of South Africa; therefore, by definition most organic compounds could be considered as ECPs.

2.7.2 Summary of the Water Resource Classification Procedure within South Africa

The WRCS relies upon seven steps for the sake of ensuring a logical and impartial process. A summary of these steps is included in this report due to the significance of understanding how the South African government conceptualises and endeavours to conserve, develop, utilise and manage water resources (especially rivers) and other forms of surface water within South Africa. The WRCS will also be used during and form part of the GIS process to identify potentially vulnerable areas and areas that might be potential pollution hotspots.

The initial step of the WRCS deals with a description of the existing condition of the water resource. This is done firstly by analysing and describing the areas surrounding and relying upon the specific water resource in terms of their social and economic activities. Ascertaining the socio-economic and ecological value of the water resource constitutes a major part of the first step of the WRCS. This includes succinctly describing the condition, needs and reliance on the specific water resources of the surrounding societies.

The combination of the value and the condition of the water resource is the purpose behind Step 2 of the WRCS. Information surrounding both socio-economic and ecosystem aspects considered. An analysis of how the socio-economic circumstances of the water body are influenced by ecological characteristics is then conducted. Various scenarios surrounding these ecological influences are then scored and weighted for further examination.

Ecological benefit and service analysis is central to Step 3 of the WRCS. The water quality required for the sustained optimal environmental yield and benefit is determined within this step. The correct extrapolation of data that pertains to any proposed, implemented or existing management procedures surrounding the water body is vital for this step to be conducted efficiently and correctly. This extrapolated data is then summarised and conveyed in the form of tables for every conceivable relevant environmental category. In so doing, the changes to the environmental sector can be accurately measured and communicated, which is what the concluding phase of Step 3 of the WRCS consists of.

Initial and environmentally sustainable baselines surrounding the use and proposed management actions of the water body is what Step 4 in the WRCS process seeks to identify. As such, this step is also concerned with the ideas surrounding the current and future use and exploitation of the water body. Considerations surrounding any managerial inputs surrounding the water body must also serve to inform the process of Step 4.

The required compromises between the socio-economic aspects and the environmental considerations are jointly dealt with by Steps 5 and 6 of the WRCS. These steps result in a decision surrounding the proposed management class, the various reserves surrounding the relevant water body, as well as the quality requirements thereof. Steps 5 and Step 6 are vitally important for anyone interested in, or reliant on the water body, as it is where the nature of the compromise between socio-economic and environmental considerations are made. These steps thus largely determine the future condition and state of the water body.

Step 5 is quite a lengthy process as it consists of seven separate objectives that must be completed. The first is to construct a model to determine the environmentally sustainable yield that can be extracted from the water body. This initial process does not include data on the consumption of water from the water body as this data is analysed in a separate second step. The important environmental impacts are summarised and communicated in a report. This report contains information surrounding any expected or predicted alteration surrounding the water that is available for use in all sectors and what the result of the altered water availability will be on socio-economic development and environmental sustainability. After further analysis, the proposed management actions are presented to all relevant stakeholders who will then provide input for further analysis that will ultimately inform the selection of a management plan. Step 6 of the WRCS comprises stakeholder consultation and feedback.

The decision surrounding the selection of the proposed management action will be communicated to the public via publication in the Government Gazette. This process is what forms the backbone behind the seventh and final step of the WRCS process. This publication is initiated when the summary of the Integrated Water Resource Management is submitted to the relevant competent authority. This authority will then be responsible for publishing the management plan in the Government Gazette, including all the relevant information surrounding the selection of the management plan, and all the socio-economic and environmental conditions, considerations and information surrounding the relevant water body. The WRCS process is then concluded by the application and monitoring of the management programme.

2.7.3 American Clean Water Act

As an example of international water quality legislation as it pertains to ECPs, the American Clean Water Act is briefly discussed for comparison purposes. This Act aims to ensure water quality is of a sufficient level to promote all forms of aquatic life. Ensuring water is safe for use by other animals, humans and for human leisure activities also forms part of this Act (Lopez, 2010). The Act strives to prevent and mitigate water pollution on every level that it can potentially occur, and to uphold the quality guidelines that are set forth by the National Recommended Water Quality Criteria (Lopez 2010).

Publishing data on water quality improvement or sustainment measures, and reviewing water quality standards at all levels of government, are enforced by the Act and the EPA is responsible for the implementation and enforcement thereof (Lopez, 2010). The universal application of the Act throughout all the states that constitute the USA is governed by section 304 of American Clean Water Act. This section also contains information surrounding the standard at which the country's water should be for a specified use. The conservation and protection of the water bodies of the country against any form of degradation is also set forth in this Act (Lopes, 2010). Areas that require more stringent water quality protection can be identified by the states themselves, following which the state can then implement any reasonable water quality guidelines specific to these areas (Lopez, 2010). Any water resource that fails to meet the minimum quality requirements as set forth by this Act must be located and identified by the state within which it is found. The state is then forced to institute a limit for the total maximum daily loads as it pertains to further or continued pollution of the relevant water resource (Lopez, 2010). This enables the state to monitor and to a certain extent control the quality of its water resources.

As is the case with the South African National Water Act, the American Clean Water Act mandates that it must be updated to ensure the continued incorporation of novel scientific information. This mandate is described in section 304 of the American Clean Water Act (Lopez, 2010). Failure to incorporate modern scientific findings in these laws will lead to a scenario where the law becomes entirely outdated and to a large extent irrelevant. It can also lead to hazardous scenarios where humans and the environment alike are exposed to danger without their knowledge and without sufficient legal protection. This means that incorporation of novel scientific findings and data should be seen as an obligation of governments globally. The EPA plays a central role in efforts to maintain the quality of American water bodies. Despite the threat that outdated laws can entail, the EPA's criteria surrounding water quality has not been modernised to incorporate any new information surrounding EDCs or ECPs. This is despite the known risk that many of these substances can entail, such as endocrine disruption and the potential to cause cancer (Lopez, 2010). It is worth noting that there are numerous other laws, for example, the Safe Drinking Water Act and the Toxic Substances Control Act, that aim to control and minimise human exposure, and protect the environment as a whole and all the organisms that live in it from harmful substances including (Lopez, 2010). These acts nevertheless fail to specifically mention or provide for EDCs and ECPs.

In order to determine which ECPs were of most importance in Gauteng, South Africa, a prioritisation study was undertaken to develop a ranked list of ECPs. In addition, maps were generated to determine potential surface water sampling sites as detailed in Chapter 3.

3 Prioritisation

By Fritz Petersen[4], James Dabrowski[5] and Patricia Forbes[6]

3.1 Objective of This Chapter

The purpose of this portion of the study was to identify relevant ECPs that could be found in South African water bodies. These ECPs were prioritised based on multiple aspects including toxicity, quantity of use, environmental persistence, relevant physicochemical data, and local (or global) prevalence to determine which of them are of greatest concern specifically to South Africa. Risk maps identifying areas of greatest concern in the province of Gauteng were created using GIS. The sites were identified by determining potential sources of ECPs including hospitals and clinics, sewage treatment plants and areas with high population densities. Water sources that have the highest probability of containing the relevant ECPs were thereby determined. This information will inform future monitoring campaigns for ECPs in Gauteng and thereby relates to the application of the fluorescence sensor technology to real environmental samples.

3.2 Risk Assessment

The procedure for the assessment of the human health risk of a substance normally consists of comparing the exposure level(s) to which the population(s) are exposed or are likely to be exposed with the exposure level(s) at which no toxic effects are expected to occur (Joint Research Centre, 2003). If all the required data is available, a risk assessment is conducted by comparing the exposure level, the outcome of the exposure assessment, with the no-observed adverse effect level (NOAEL), the outcome of the dose-response assessment. If sufficient data is not available to establish a NOAEL, a comparison between the exposure level and the lowest observed adverse effect level (LOAEL) can be used to gauge risk (Joint Research Centre, 2003).

The precise ways in which many ECPs react and change in the environment and the concentration at which they will start eliciting effects in the environment remain largely unknown. Modelling programmes and predictive software that can estimate these values do exist although they are often lacking and cannot provide data on the oestrogenic effects of these chemicals. The environmental risk or hazard posed by some ECPs will thus be characterised with high uncertainty (Diamond et al., 2011).

A thorough risk assessment process in relation to both human health and the environment typically entails three main actions, namely (Joint Research Centre, 2003):

1) An assessment of the effects.
2) An assessment of potential exposure.
3) A risk characterisation.

Assessing the effects of a chemical (1) can be subdivided into two stages, namely:

- Hazard identification (identification of the adverse effects that a substance has an inherent capacity to cause).
- Response or effects assessment (estimation of the relationship between dose, or level of exposure to a substance and the incidence and severity of an effect).

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Potential exposure assessments (2) are an estimation of the concentrations/doses to which human populations or fauna and flora and ecosystems in general are or may be exposed. The final part of the process, namely, risk characterisation(3) is an estimation of the incidence and severity of the adverse effects likely to occur in a human population or any part of an ecosystem due to actual or predicted exposure to a substance (Joint Research Centre, 2003).

Exposure can be understood as external exposure that can be defined in a variety of ways dependent upon the type of exposure. Types of exposure can include the amount of substance ingested, the total amount in contact with the skin (which can be calculated from exposure estimates expressed as $mg \cdot cm^{-2}$ or $mg \cdot cm^{-3}$), or either the amount inhaled or the concentration of the substance in the atmosphere (Joint Research Centre, 2003). Anthropogenic exposure to substances can occur in a variety of ways including from their workplace to indirectly via the environment. Indirect exposure of humans via the environment may occur by consumption of food (fish, crops, meat and milk) and drinking water, inhalation of air and ingestion of soil.

Exposure can be considered as single events, or a series of repeated events, or as continuous exposure. The duration and frequency of exposure, the routes of exposure, human habits and practices, as well as the technological processes need to be considered when determining the risk that a chemical poses (Joint Research Centre, 2003). Additionally, the spatial scale of the exposure (e.g. personal/ local/regional level) can also greatly influence how hazardous a chemical is. Thus, it has to be considered (Joint Research Centre, 2003). It is worth noting that when assessing the levels of chemicals in the environment, previous releases of the chemical into the environment need to be considered. These releases can give rise to a pre-existing background concentration due to their cumulative effect in the environment, which can cause inaccuracies during *in situ* data gathering.

The environment may be exposed to chemical substances during all stages of the chemical's life cycle from production to disposal or recovery (Joint Research Centre, 2003). For each environmental compartment (air, soil, water, sediment) potentially exposed, the risk assessment procedure should account for the following stages of the life cycle of a substance: production; transport and storage; formulation (blending and mixing of substances in preparations); industrial/professional use (especially large-scale use); private or consumer use; service life of articles; waste disposal (including waste treatment, landfill and recovery) (Joint Research Centre, 2003).

3.3 Approaches to Prioritisation

Expert opinion and project specific parameters can often heavily influence scientific inquiries into ECPs. This is because both expert opinion and project specifics commonly dictate which chemicals will be investigated and may form the basis or starting point of these studies. Occurrence information, toxicity, endocrine disruption capability, biological persistence (half-lives in the environment) and information surrounding the popularity and widespread use of these chemicals formed the backbone of the parameters used to target and monitor ECPs in a recent study by the United States Geological Surveys (USGS) (Diamond et al., 2011). Expert opinion along with traditional analysis methods formed the basis of a similar study to that of the USGS that was conducted by the US EPA. This study was concerned with PCPs and their effect in and on the environment (Ramirez et al., 2009). These examples clearly illustrate that even a developed nation like the USA at the forefront of science and technology still mainly relies on expert opinion when targeting and monitoring ECPs, and has not developed an unbiased prioritisation methodology for evaluating these chemicals.

The concentration of multiple pharmaceuticals and chemicals known to have endocrine-disrupting capabilities were analysed in a recent study on the water bodies of America (Benotti et al., 2009). The authors considered different criteria during the selection of the chemicals that were analysed. Pharmaceuticals that have greater toxicity and a higher general potency were emphasised. Therefore, medication that could only be obtained by means of a prescription were central to their study.

Anthropogenic toxicity scores, measured occurrence data and interest in the chemical from the general populace formed part of the factors that were considered by the researchers alongside the popularity of the chemicals (that was measured by occurrence data and volume of use).

The study implemented a similar screening process for EDCs. Parameters seen as important included: the reported magnitude of endocrine effect, occurrence, toxicity, biological persistence, accumulative potential, how the chemical changes and functions, and concern towards the chemical expressed by the general populace. The availability of analytical methods was a consideration for both pharmaceuticals and EDCs. Expert opinion did, however, still form the basis for this research, which detracts slightly from the concise and thorough nature of the rest of the methodology.

Environment Canada has developed a framework for classifying and ordering chemicals and the potential hazards posed by them that constitute the Domestic Substances List. The accumulative potential, half-life and toxicity as it pertains to non-target animals are the factors that inform the assessment procedure (Arnot & Mackay, 2008). It is thus a hazard-based approach seeing as actual occurrence information surrounding the chemicals from the Canadian environment is not considered. Persistence of the chemicals are assessed based on their half-lives in water, where an aquatic half-life of 182 days or more is seen as a concern. The partition coefficient is used to ascertain whether a chemical will accumulate in the environment, where chemicals are included that have a minimum partition coefficient value of 5 (log P_{ow}) (Arnot & Mackay, 2008). Bioaccumulation and bioconcentration factors can also aid in informing this process where a value of 5000 is the cut-off value for assessing persistence (Arnot & Mackay, 2008). Toxicity of the chemicals are analysed according to acute toxicity (LC_{50}) as well as chronic toxicity when data is available. As previously mentioned, chronic toxicity is the far more useful measurement for ECPs due to the high acute toxicity values possessed by most of these compounds.

Before the commercial production of a novel chemical can commence, the substance must go through stringent analysis and assessment according to the EPA and in accordance with the Toxic Substances Control Act. The environmental fate and consequences must be diligently analysed and reported on during this mandatory analysis. Toxicity analysis is only conducted when a chemical has a biological persistence that is seen as significant, which is often the case when it can remain present in the environment for a time period of more than two months (Diamond et al., 2011). A chemical’s capacity for accumulation in the environment can also lead to extended analysis of its toxicity if the accumulation or bioconcentration factors are in excess of 1000 (Diamond et al., 2011). Substances that feature a persistence period in excess of half a year or that feature a bioaccumulation factor in excess of 5000 will not be allowed to be produced until a thorough analysis of their toxicity data has taken place (Diamond et al., 2011).

Once again, this approach (as with the Canadian one) does not calculate risk but is focused on the hazard that is posed by these chemicals. This is because this approach does not rely on measured environmental occurrence data. Other research has focused on analysing the whole spectrum of environmentally relevant constraints such as persistence, bioaccumulation and toxicity which is known as the PBT approach. Major environmental agencies and governing bodies that follow this approach includes the Oregon Department of Environmental Quality, certain divisions with the EPA, and even the United Nations (refer to the Stockholm Convention on Persistent Organic Pollutants: www.pops.int/).

The PBT approach is used by the Canadian Domestic Substance List as well as the Toxic Substance Control Act of the USA (Diamond et al., 2011). This approach allows for a regulatory framework to be constructed for these chemicals. Objections surrounding the use of the PBT methodology for the purpose of analysis of ECPs have not been uncommon for all interested and affected parties that rely or use water bodies. Substances that feature relatively low production numbers, such as many pharmaceuticals, are often overlooked for analysis. It should, however, be mentioned that the risk that is posed by an ECP often does not share a direct relationship with its production volume or frequency of its usage. ECPs that are more toxic and generally more potent in their effect towards aquatic ecosystems are often of greater concern (Ankley et al., 2007). Looking purely at a chemical's environmental persistence can provide a skewed interpretation of the amount of exposure occurring in the environment – especially if these chemicals are constantly being released into the environment, as is the case with the discharge of effluent water. The constant supply of these chemicals into the environment will thus serve to override their inherently short persistence times and half-lives. Traditionally applied toxicity analysis, especially as it pertains to the endpoints of chemicals, has been reported to be inadequate for accurately analysing the potential consequences of both EDCs and certain ECPs (Ankley et al., 2007). Flexibility during the process of accurate risk and hazard-orientated prioritisation methodologies are essential when it comes to EDCs and ECPs. The flexibility referred to alludes to the incorporation of both *in situ* and predicted exposure concentrations.

Environment Canada commissioned a study in 1999 with the purpose of compiling a priority list of EDCs (Haarhoff et al., 2015). The study was started by compiling a list of suspected EDCs from a multitude of databases and experts. A total of 564 compounds were identified during this first step for further consideration. These initial compounds were then subjected to three successive screening steps:

- The first step of this study was concerned with the production volume (more than 1000 tonnes per year) and persistence of the chemicals with the goal of identifying the chemicals that had a greater possibility of human exposure (Haarhoff et al., 2015). The persistence of the chemicals was determined by their biodegradability [attained from quantitative structure–activity relationship models (QSAR)] or from the ultimate degradation (complete mineralisation) time (Haarhoff et al., 2015). After this initial step 146 chemicals remained.
- The second step in this study focused on analysing the endocrine-disrupting potential of the remaining chemicals (Haarhoff et al., 2015). They were divided into three categories based on their endocrine-disrupting potential as determined by a panel of experts (Haarhoff et al., 2015). Category 1 consisted of chemicals that had at least one study providing evidence of endocrine disruption. Category 2 consisted of chemicals that had potential for endocrine disruption. Category 3 compounds were proven not to cause endocrine disruption or suffered from a lack of available data, thus could not be categorised.
- The final step in this study was focused on the concern for exposure and was also based on expert knowledge and was conducted by a panel of experts (Haarhoff et al., 2015). The remainder of the chemicals were classed as high (denoting chemicals expected to incur exposure to humans and wildlife and were considered to be persistent and bioaccumulative), medium (denoting chemicals that could incur exposure to humans and wildlife but are biodegradable and thus cannot bioaccumulate), and low (denoting chemicals that feature no human or wildlife exposure).

Evaluating some of the chemical prioritisation schemes currently in use raises important points regarding the development of a more inclusive and useful prioritisation framework to analyse water resources. Prioritising chemicals commonly used and that enjoy widespread application according to their toxicity and environmental considerations has limited usefulness. This is due to two primary reasons; the first of which is that there are many important and potentially hazardous chemicals that will be excluded from the initial list since they are used less frequently. The second reason that limits the usefulness of this approach centres on the fact that there is uncertainty around the likelihood that many of these chemicals have of actually entering the environment and water bodies. The European Union has acknowledged these shortcomings and they have also stated that chemicals that are manufactured in lower quantities need to be included with specific focus on chemicals known or suspected to possess endocrine-disrupting capabilities (Petersen, 2007).

More than 40 000 organic chemicals have been identified as ECPs (Diamond et al., 2011). ECPs are often poorly characterised in terms of their presence in aquatic environments and their potential effects on aquatic wildlife and humans, although some have been proven to be harmful to fish, other animals, and even humans (Diamond et al., 2011). Clarity needs to be found over which of these chemicals pose the highest risk. The creation of a comprehensive list of potentially harmful ECPs would result in an unmanageable list of more than 40 000 substances (Diamond et al., 2011), thus some type of prioritisation of these chemicals is needed to identify the chemicals of greatest concern to provide focus to monitoring campaigns. Studies focusing on ECPs and their prioritisation typically follow one of three approaches, namely, hazard-based, hazard-based combined with persistence and bioaccumulation potential, or PBT(Diamond et al., 2011).

3.3.1 Approach #1

The risk-based approach is usually based on the proportion of the maximum observed concentration of a specific ECP to its predicted endpoint, based on either chronic toxicity or oestrogenicity effect (Diamond et al., 2011). Oestrogenic activity has become a routine consideration and a standard indication of sub-lethal effects as many chemicals are not highly toxic. These chemicals can, however, still have sub-lethal effects that may not be detected using standard toxicity tests (Diamond et al., 2011). This approach is easy to implement and communicate and it gives an idea of what is happening in reality as it relies on *in situ* data measurements. Although this approach is relevant to predicting ecological risk, it only makes provision for ECPs found in water bodies and does not consider the production data of chemicals (Diamond et al., 2011).

A risk-based approach can see widespread application in organisations and studies that seek to evaluate the environmental risk posed by ECPs that are based on *in situ* measurements that have been gathered from multiple monitoring exercises and research inquiries. Due to the dependence of this approach on measured occurrence data, it can prove an invaluable starting point for sustained monitoring studies. Site-specific research can also benefit from this approach as measurements obtained from that specific site can be included in the calculations and can thus become more relevant and accurate.

In the study by Diamond et al. (2011), three calculations were used to determine risk, which are:

Calculation 1:

$$Risk\ value = \frac{Highest\ measured\ concentration}{Minimum\ chronic\ toxicity\ limit}$$

Calculation 2:

$$No\ effect\ endocrine\ activity\ risk\ value = \frac{Highest\ measured\ concentration}{Predicted\ no\ effect\ concentration}$$

Calculation 3:

$$Endocrine\ activity\ risk\ value = \frac{Highest\ measured\ concentration}{Predicted\ effect\ concentration}$$

ECPs for which the *no effect endocrine activity risk value* calculations equated to less than 1 were described as unlikely to be present in water bodies at quantities sufficient enough to cause significant disturbance to the endocrine systems and reproductive capacities of organisms in the environment. These ECPs will then be considered as unimportant in terms of the risk they pose for endocrine disruption. Chemicals that score higher than 1 (seen as important chemicals regarding endocrine disruption capabilities) for the *endocrine activity risk value* can thus be considered to occur at sufficient environmental concentrations to cause harm to the reproductive systems of organisms. Chemicals that score more than 1 for Calculation 2 as well as less than 1 for Calculation 3 are seen as rather ambiguous and as such are treated with a great degree of uncertainty. This is because of the possibility for these chemicals to cause reproductive harm and to disrupt the endocrine systems of organisms cannot be accurately gauged.

3.3.2 Approach #2

Approach number two assigns scores to a chemical’s characteristics in terms of its bioaccumulation potential and half-life in water (persistence in the environment), which is then used to calculate a risk-based quotient (Diamond et al., 2011). As this approach depends largely on environmental occurrence information, it relies on samples obtained from the environment. These scores ranging from 1 to 3 are assigned based upon whether the chemical has scored relatively low, medium or high in that category and thus how much of a threat it poses. This approach sees widespread application and its scoring system is also commonly used by agencies including the US EPA and the Canadian Domestic Substance List (Diamond et al., 2011).

The scores assigned to the various relevant factors for every ECP are then added together to create an estimation of how great a risk all the chemicals pose. The maximum score that can be achieved is 9 (3 + 3 + 3) and can only occur if a chemical has a high score assigned to it for each individual factor under consideration. A score of 7 or more means that the chemical is considered of high importance as it poses a significant potential threat and as such it will be seen as a priority ECP. Any chemical that has achieved the maximum score of 3 for any single factor will also be flagged as a potential priority ECP.

More substances are commonly analysed using this approach due to the less stringent initial requirements a chemical has to meet to be included in the target analyte list. Analyses can also be seen as more thorough since biological accumulation and environmental persistence are considered in addition to the risk-based quotient. A total score of 9 will lead to a chemical being considered to be a priority due to three predominant reasons. The first reason is that they have been found in water bodies at quantities that surpass the lower limits of either their toxic or endocrine-disrupting thresholds. Secondly, they can potentially undergo bio-magnification and thus have devastating consequences for organisms that are found in higher trophic levels. The third reason is that they can remain prevalent and present in the environment for extended periods of time owing to their long half-lives in water and thus inferred persistence. Environmentally concerned companies or individuals can find this approach useful – particularly those who feel that the incorporation of occurrence data is absolutely essential to the prioritisation of ECPs. This is due to the thorough estimation of risk that is applied with this approach. It can also prove valuable to site-specific analysis as *in situ* data that has been acquired from the site itself can be incorporated into the approach leading to the results becoming more specific and relevant to that site.

3.3.3 Approach #3

The PBT prioritisation approach is similar to the hazard, persistence and bioaccumulation approach, except that it relies on predicted toxicity values obtained from software and does not require *in situ* data measurements (Diamond et al., 2011). Mathematical models are used to estimate or predict ecotoxicological effects. ECOSAR is the most popular QSAR model that was developed for this purpose (Sanderson et al., 2004). Despite the models often having serious drawbacks, such as an inadequate coverage of the various structures of pharmaceuticals, these programs are used to estimate pharmaceutical baseline toxicities (Sanderson et al., 2004). Although models are helpful in estimating potential toxicity or the behaviour of a compound in the environment, they cannot replace *in situ* measurements or controlled tests. The PBT approach can lead to an undesired scenario where an ECP is scored as a high priority but may actually never occur at concentrations that would cause chronic toxicity or endocrine disruption in the environment.

ECPs prioritised by Approach 3 cannot be considered to be risk-based since no occurrence information is considered. This means that it focuses primarily on the estimation of the potential hazard that these substances can pose. It is also not reliant on *in situ* data measurements as it can use software that estimates the toxicity, persistence and bioaccumulation of chemicals based on their chemical structures. A total score of 7 is still seen as a priority chemical when the three categories are summed. People who are concerned with the theoretical hazard that ECPs can pose will find this approach very useful. This includes studies that seek to determine whether chemicals that are not currently monitored as part of ECP monitoring programmes (due to lower manufacturing or occurrence data) should perhaps start being analysed and monitored due to the theoretical risk that they pose.

The advantages and constraints of each approach must be understood if ECPs are to be prioritised using one of them. To this end, a summary of these approaches has been included as Table 1, which originated from the study by Diamond et al. (2011).

Table 1: Prioritisation approaches for ECPs (based on Diamond et al., 2011)

Approach	Summary	Advantages	Constraints
1	Risk-based calculation that used occurrence data and effect concentrations. Focuses on ECPs having the most frequent use and those that have the highest occurrence in water bodies.	Straightforward application and ease of data interpretation. Analysis of results does not require specialist knowledge. Accuracy can be substantiated by using data obtained by scientific inquiry. Can determine actual risk and not just theoretical risk.	Occurrence information can be hard to come by and as such accumulating all the required information for this approach can be time-consuming. ECPs lacking occurrence data (which is common) cannot be analysed by this approach.
2	Similar to Approach 1 as it also relies on occurrence data. Also concerns itself with bioaccumulation and persistence and strives to combine this with occurrence information to predict possible risk.	Accuracy can be substantiated by using data obtained by scientific inquiry. Can determine actual risk to an even greater extent than Approach 1 as ECPs with lower occurrences can still become a priority. Numerous applications from an environmental health perspective.	Occurrence information can be hard to come by and as such accumulating all the required information for this approach can be time-consuming. ECPs lacking occurrence data (which is common) cannot be analysed by this approach.
3	Typically uses predictive software to calculate toxicity, bioaccumulation and persistence. These predicted values are assigned a weight and are combined to calculate potential hazard. Largely theoretical.	Uses a traditional approach and can give great insight into the theoretical hazard that a substance can pose. Data collection can also be done more rapidly.	Substance seen as a priority may never occur at environmentally relevant concentrations and might thus not be reflective of actual risk. Accuracy of software used to make calculations might also be a concern.

Environmental programmes in the USA, Canada, and Europe tend to focus on the production data of chemicals when determining the risk that they pose (Diamond et al., 2011). The reasoning is that chemicals that are produced in larger quantities are more likely to reach surface waters, thus they present a greater risk than chemicals produced in smaller quantities. This approach fails to consider that some chemicals are unlikely to enter surface waters. The most threatening ECPs from a health impact perspective are not necessarily produced in high volume and are therefore not on their lists.

There are four main issues that hamper the development of more effective monitoring efforts (Diamond et al., 2011):

- Lack of understanding how *in situ* measurements of ECPs translate into ecological risks.
- Different definitions for ECPs leading to studies that focus on varying sets of chemicals that they identify as ECPs.
- The trace amounts at which ECPs typically occur in the environment means that detecting them is often difficult if traditional analytical methods are used.
- Lack of toxicity data and when available it is limited to species-specific data.

A thorough prioritisation process should include at least some form of risk assessment so that ECPs posing the greatest possible threat to the environment can be located and monitored as high priority chemicals. An accurately integrated evaluation of the risk assessment information is required to accurately reflect on the existing condition as it relates to the occurrence of ECPs to ensure that an accurate, meaningful and representative prioritisation process is established (Diamond et al., 2011).

ECPs can be similar to “traditional” pollutants, for example, heavy metals or ammonia, when considering the risk they pose to the environment, as they occur with more obvious stressors that could mask subtle sub-lethal effects (Diamond et al., 2011):

- The anatomical and physiological impacts on separate organisms within a freely breeding population can possibly be undetectable at the community or even at the population level.
- Short environmental and specifically aquatic half-lives do not necessarily mean that the chemical does not have pseudo-persistent characteristics. These characteristics and their associated effects can be the direct result of the uninterrupted release of the chemical into water resources.

ECPs do nevertheless vary from traditional pollutants in that:

- The precise modes of action that are characteristic of various ECPs can cause tremendously diverse impacts on dissimilar organisms and/or life stages.
- The effect of ECPs on future generations might be more severe than on the current generation due to the strong propensity for endocrine disruption that many of them possess. Many ECPs are also not extremely toxic and may thus not kill organisms they come into contact with directly.
- Many ECPs feature unidentified modes of action and the relationships between the concentrations they are present at and the effect they illicit are not well understood. This is particularly true when they occur at low concentrations.

These differences mean that the conventional and widely applied risk-based method for prioritising pollutants might require certain modifications in order to assess ECPs in a more representative and applicable manner (Diamond et al., 2011). It appears that basing an ECP prioritisation framework on reasonably representative occurrence information will likely be the most useful approach (Diamond et al., 2011).

It should, however, be noted that the use of *in situ* measurements of occurrence data and manufacturing volumes only are not adequate for the prioritisation of ECPs as this data must be combined and analysed in conjunction with pertinent information surrounding possible environmental effects and their propensity for accumulation and persistence within the environment and all the organisms that live within it. The combination of these characteristics is required to thoroughly prioritise ECPs in terms of potential ecological risk. Analysing toxicity and potential for endocrine disruption can been seen as the most pertinent of environmental or effect endpoints during the screening for or prioritising of ECPs (Diamond et al., 2011). The difficulty in determining and quantifying the synergistic effects for mixtures of compounds is also worth noting.

3.4 A Previous South African Approach

In a South African prioritisation study completed in 2009 (Ncube, 2009), the following steps were followed to first form a comprehensive list of ECPs and, secondly, to prioritise these chemicals so that a decision could be made surrounding their removal from South African water bodies. The initial step of this study was to select a preliminary list of chemicals that would be refined and then later analysed more intensely (Ncube, 2009). Various databases were consulted and all ECPs that are either restricted or banned in specific areas were also included. Stakeholder consultation also took place and experts were also given the opportunity to provide insight and opinions that validated the chemicals considered. This resulted in the elimination of unnecessary chemicals and overall refinement of the group of chemicals that were analysed in more detail (Ncube, 2009).

The next step was the further verification of the list by considering occurrence information and the potential of the chemicals to cause negative health effects (Ncube, 2009). A literature review was used to accomplish this. Information surrounding the physicochemical properties of the various ECPs was also gathered for use in the prioritisation process once a final analyte list of chemicals was decided upon, as these characteristics can be used to predict the fate of an ECP once it enters the environment (Ncube, 2009). A PBT approach also formed part of the prioritisation process (Ncube, 2009). This step was centred around gathering supplementary data on ECPs to assist with analysis. Water quality monographs were used to present the data and illustrate it in a clear way (Ncube, 2009).

The next step in this study was the taking of fish tissue, sediment and water samples for analysis to determine if and where ECPs occurred in water bodies (Ncube, 2009). Analytes present in the samples were included in the final list of compounds that were prioritised and analysed further. This list was also subjected to the approval of stakeholders, experts and industry leaders (Ncube, 2009).

Once a final list of ECPs was decided upon, the actual prioritisation took place that divided the chemicals into short term, medium term and long term based on the need for further priority analysis within South African water bodies (Ncube, 2009). The ECPs that were placed onto the short-term list were viewed as chemicals that warranted the highest concern and required further continued monitoring.

For an ECP to be placed in the short-term category, it had to possess the ability to cause negative health impacts to humans (Ncube, 2009). Chemicals that cause water quality problems including problems surrounding the taste and smell of the water were also placed on the short-term list, as were chemicals that cause an increase in risk perception among the general population. If an ECP had poor removal rates using traditional water treatment processes or if the regulation of the chemical was enabled due to pre-determined drinking water standards, it was included in the short-term list. The short-term list was rounded out by including ECPs that were known to occur within South African water bodies and especially those that were found in drinking water (Ncube, 2009). Finally, stakeholder consultation was completed again before the final list was decided on (Ncube, 2009).

3.5 Methodology Employed in This Research

The methodology for this project consisted of two components. The first was concerned with obtaining a relevant and inclusive list of ECPs that were to be prioritised after relevant data had been collected. These ECPs were prioritised to gain a ranked list of priority ECPs relevant to South African conditions and around which future research efforts within South Africa should be focused. The resultant list will also be a suitable starting point for any agencies wishing to start monitoring ECPs and for scientific studies that focus on ECPs within a South African context. The second part of the methodology was centred on using GIS to locate areas that are likely to be the most vulnerable to ECPs and areas that might be potential ECP hotspots. These identified areas can be used to assist with the identification of suitable sampling sites for monitoring agencies wishing to monitor ECP concentrations in vulnerable areas and for future studies surrounding ECPs.

3.5.1 Prioritisation methodology

The list of compounds that was subjected to the process of prioritisation and ranking was obtained after a thorough literature review of multiple sources. The literature included both local and international studies, to ensure the list was as comprehensive and inclusive as possible. Specific focus was given to other prioritisation studies regarding ECPs and all the substances that were concluded to be priority substances from these studies were included in the initial list of compounds. Thus, this study can be seen as a second-tier prioritisation and ranking study as the initial list of compounds were already found to be priority substances based on other scientific studies. This study thus seeks to refine a list of ECPs that have already been found to be priority pollutants and being known to be potentially hazardous in certain regions. It also strives to make this refined list specifically applicable to South African circumstances and conditions.

The method followed for this project was partway between Approach 2 and Approach 3 (as detailed in Chapter 2). The initial list of compounds was first split into two groups, namely, those with occurrence or usage data available and those where neither usage nor occurrence data was available. If there was some form of usage or occurrence data available for a compound, a risk-based approach could be employed, and the actual threat posed by the substance could be calculated. This was done in an attempt to ascertain what the actual potential risk was that these compounds posed to both human and ecological health. As previously mentioned in the literature review, accurate information on usage or occurrence of ECPs is not always readily available and this is particularly true in the South African setting.

When data surrounding occurrence or usage was not available for a given compound, it was classed in the list of compounds that lack this data. These compounds were prioritised via a hazard-based approach as the theoretical threat they pose could be calculated, but without occurrence or usage data an actual risk-based approach was not possible. This was the case as a compound could hold a large theoretical threat based upon its toxicity, persistence and physicochemical data, but it could perhaps never occur in high enough concentrations in the environment to have any effect or cause any significant harm. The list of compounds analysed and placed within the group of compounds that lack data can always be moved over to the list of compounds that have occurrence or usage data as this data becomes available in the future. Thus, a compound can always be analysed according to the risk-based approach as more usage and occurrence data on the relevant compound becomes available.

For the purpose of prioritisation and analysis, the list of compounds was analysed within three tables, each of which focused on a different aspect that is pertinent to the potential risk or hazard that is posed by the ECPs. The tables focused on (1) physicochemical data, (2) occurrence data and the efficiency of WWTPs at removing the compounds, and (3) PBT, respectively. A secondary analysis on information surrounding the prescription and import of these substances can be added when available and ordered to sort the substances from the most frequently used to the least frequently used. It also analysed studies that investigated the efficiency of conventional WWTPs at removing ECPs to determine how effectively these substances are being removed from water resources. The third table was centred on determining the toxicity of the ECPs as well as their affinity to bioaccumulate within organisms as well as analysing their ability to persist within the environment.

The attributes and factors of the ECPs considered to be important within the PBT table and the physicochemical data table were: octanol-water coefficient, biological half-life, toxicity (LD_{50} and EC_{50}) within a standardised endpoint (rats or algae), endocrine disruption capability, volatility, and vapour pressure. These factors were considered of vital importance when determining what hazard the various ECPs posed and, thus these factors played a major role in determining the priority that would be assigned to a compound.

Physicochemical data was obtained from materials safety data sheets (MSDSs) for the compounds concerned (primarily from Sigma-Aldrich) and from studies that investigated or contained data on the toxicity, persistence of compounds. However, in instances where such information was unavailable or could not be found, chemical modelling programmes were used to gain the required information. The PBT profiler programme (www.pbtprofiler.net) was used for this purpose. These programmes predict the physicochemical, toxicity and persistence data based upon the chemical structure of the compound. The programme relies on input in the form of the chemical's name or the Chemical Abstract Service (CAS) number. These programmes have been commonly used in various studies regarding ECPs and it was the most efficient, cost-effective and reliable manner in which to acquire the data that was missing after the extensive literature review was completed. A well-known prioritisation study by Diamond et al. (2011) also relied upon modelling programmes to acquire data that was not freely available. Numerous studies prior to this also did so (Diamond et al., 2011).

During the final part of the prioritisation, the data from the various tables were combined to determine what risk/hazard was posed by the various ECPs. The first step towards achieving this was by ranking the ECPs within each table based on the relevant data that was obtained about the ECPs. Thus, every table resulted in a ranked list of ECPs for that particular set of factors. The lowest rank was assigned to the highest scoring chemical, thus the chemical that had the highest score for a particular factor, such as toxicity for example, would receive a rank of 1. This was done with the full knowledge that not inverting the ranking would provide the same result. The inverse order was used as it was thought that it made analysis after each step simpler as the most significant chemical for that specific parameter would receive the ranking of 1 instead of 168, which it would have received if the ranks were not assigned inversely.

To gain an all-inclusive rank for a particular ECP, the rank that it held within each table was simply summed together, giving it a score based on all the factors relevant to determine its potential risk or hazard. This means that compounds with lower summed rank values are of higher concern and are thus more of a priority than compounds with a higher summed rank value. The end result of this process was a ranked list of priority ECPs.

3.5.2 GIS methodology

The methodology was based on locating the river catchment areas within the Gauteng region that would be the most susceptible to contamination. Figure 2 shows an overview of the various phases that were followed to locate suitable sampling areas that could possibly contain contaminants in most vulnerable areas within Gauteng with a specific focus on anthropogenic chemicals. This was done by identifying all the protected and vulnerable areas within each of the catchments by looking at the ecosystem status of each catchment as defined by the South African National Biodiversity Institute (SANBI), identifying the number of medical facilities and WWTPs, and determining the population density of the areas.

From this information, an analysis was done to determine the catchment(s) that have the highest vulnerability and could have the most detrimental effects if high levels of contaminates were found. This analysis was completed by combining the variables listed above within the catchments using an overlay approach. Here each of the factors most likely to contribute to contamination within the catchment was analysed according to a criterion and further ranked based on risk. The combinations of variables were then overlaid to extract the catchment(s) that would be the most susceptible to contaminants. The water features within these vulnerable catchments were further extracted and maps depicting the areas that are most vulnerable and have a high probability of containing contaminants were created. Further detail is provided in Figure 2.

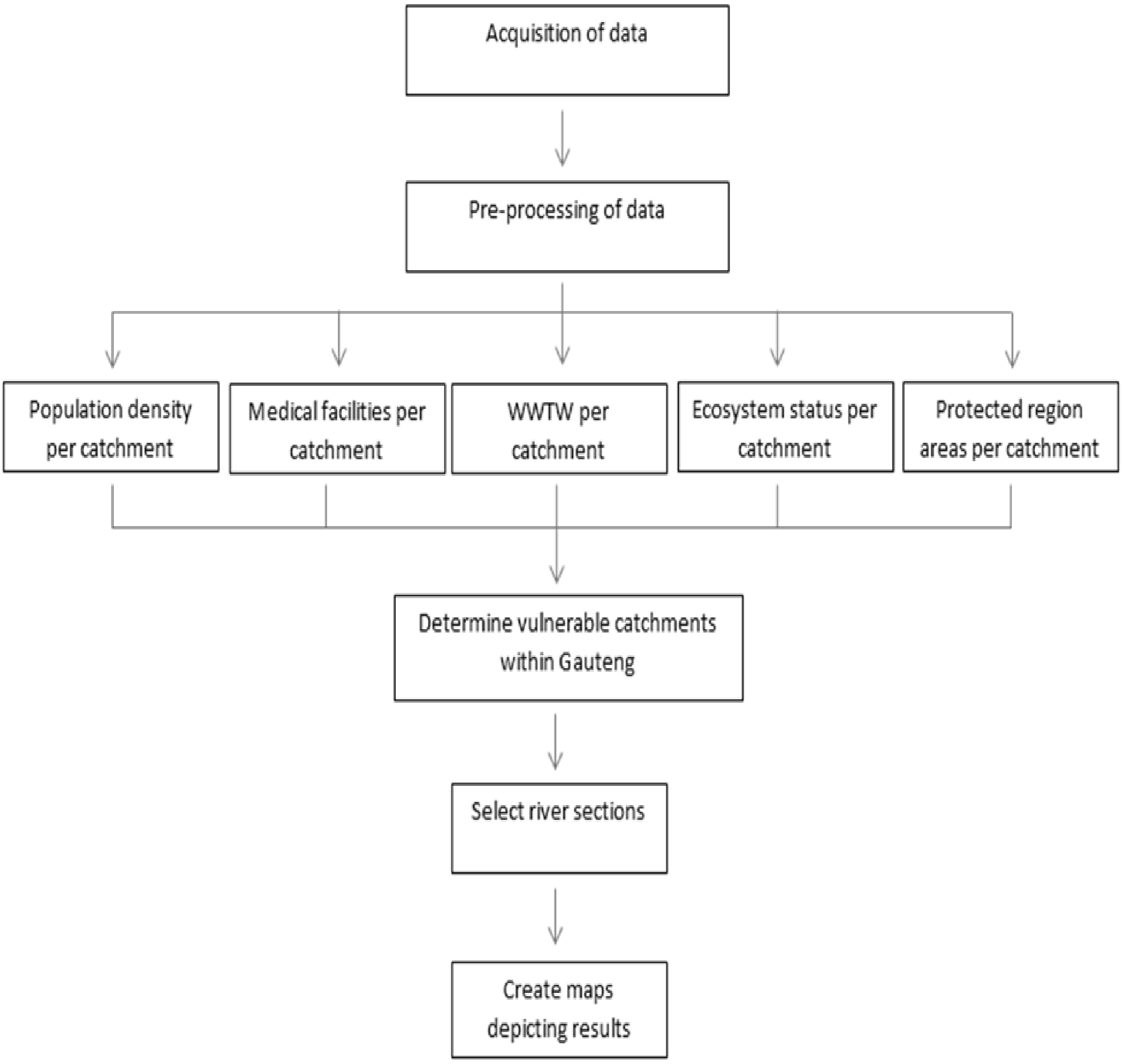

Figure 2: Overview of GIS methodology for anthropogenic substances

A similar diagram depicting the process followed for the agricultural chemicals is shown in Figure 3. The overall process was very similar. It is worth noting that population density was retained as a parameter, but for different reasons compared to its role for the anthropogenic chemicals. For the both types of chemicals, population density meant there was a greater risk of those chemicals finding their way into the water resources of the region. In the case of the agricultural chemicals, population density had a direct influence on the potential for an agricultural chemical to cause harm as regions where more people live can mean that a chemical has the potential to cause more harm than in sparsely populated areas.

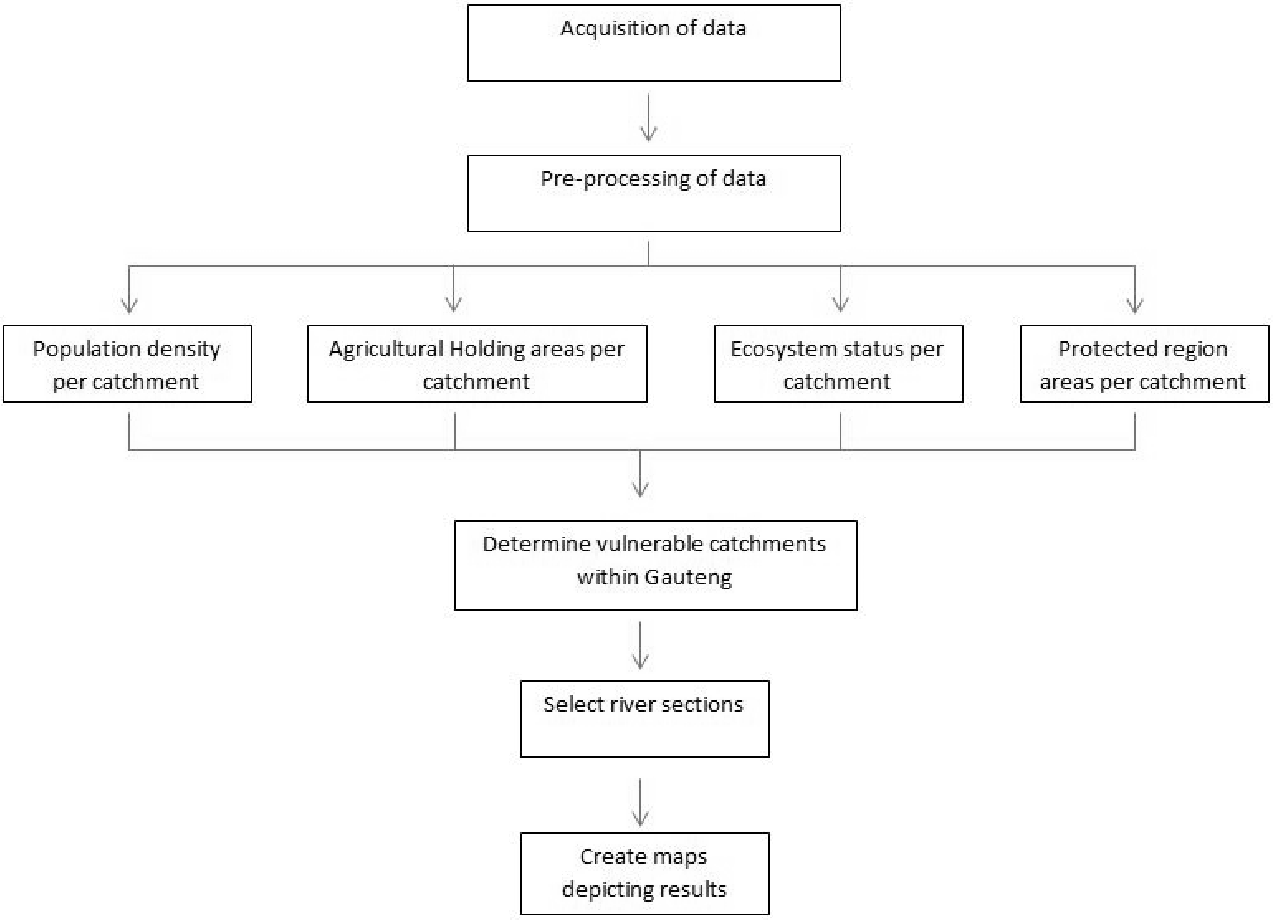

Figure 3: Overview of GIS methodology for agricultural substances

3.5.2.1 Acquisition of relevant data

For this research study, a number of spatial and non-spatial data sets were required, which are summarised in Table 2.

Table 2: Required spatial and non-spatial data sets for GIS modelling

Required data set	Format	Main purpose	Source[7]
Population density	Shapefile per ward	Used to determine which catchment would be most vulnerable	Statistics South Africa
WWTPs	Excel sheet	Locate containment points and create risk weighting	Department of Water Affairs
Medical facilities	Shapefile (points)	Locate containment points and create risk weighting	Department of Health
Protected areas	Shapefile (polygons)	Determine areas of vulnerability and contribute to the selection of catchments	SANBI
Water catchments	Shapefile (polygons)	Used as base data	Department of Water Affairs

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[7] All the data was directly requested and as such was obtained via personal communication

Required data set	Format	Main purpose	Source[7]
Water features	Shapefile (lines and polygons)	Used as base data and obtain sampling areas	MapIt and AfriGIS
Land use data	Raster data	Ecosystem status	National Geo-Spatial Information
Agricultural data	Shapefile (polygons)	To determine areas of vulnerability	National Department of Agriculture
Orientation data	Various formats	Orientation, visualisation and planning	SANBI, AfriGIS, Department of Rural Development and Land Reform

3.5.2.2 Pre-processing of data

To successfully analyse the data and select suitable sampling sites, the first step was to ascertain data interoperability between the required data sets. The data formats needed to be compatible with each other and the GIS software package. For this research project, the Environmental Systems Research Institute (ESRI's) ArcGIS software package (version 10.1) was used. The ArcGIS data interoperability function is known as Spatial ETL tool, which allows the user to do conversions between formats and a platform where the data can be analysed and visualised regardless of the format. All the data sets acquired for this study were also clipped to the Gauteng Province for faster analysis and processing except for the catchments. The full catchment boundary was needed for accurate selection; here an intersect tool was used to create a selection of the catchments that overlapped the Gauteng Province boundary.

3.5.2.3 Selecting vulnerable catchment(s) (anthropogenic chemicals)

The term "anthropogenic compounds" will be used to describe all chemicals not used during agricultural practices, such as antibiotics or PCPs. Selecting the most vulnerable catchments in Gauteng would give an indication of where contaminants would most likely have the greatest impact if found. The first step was determining the population density per catchment. The current population density data was populated per ward and not per catchment. A geostatistical analysis tool had to be used to reallocate data from one set of polygons (area) data to another set of polygons. Here a real interpolation was implemented in a two-step process; firstly, a smooth prediction surface was created from the source polygon – in this case the population per ward. This surface was then reallocated to each of the target polygons, which were the catchment areas within Gauteng. This gave an accurately estimated population per catchment.

Medical facilities and WWTPs are most likely to be the sources of contaminants due to the high concentration of ECPs used and processed by them respectively; therefore, the second step in selecting the vulnerable catchments was to locate the number of medical facilities and WWTPs per catchment. This was done using a spatial join, which joins attributes and calculates statistics based on spatial relationships. For both medical facilities and WWTPs, the count statistic indicates the number of features that were joined in the matching target area (catchments). This layer was then joined with the population density layer calculated in the previous step.

The next objective in selecting the vulnerable catchments was locating all the protected areas and areas of concern per catchment. This was completed by importing all the protected and high priority areas into ArcGIS and then using the Model Builder tool to clip all the features within each catchment. The summed area of these protected areas per catchment were then calculated by exporting the attribute table to Excel and summing the various areas for each catchment. Furthermore, a field was created in the layer with the population density attribute table and the summed area of protected areas per catchment was manually updated. The area of protected areas per catchment would give a good indication of the extent the vulnerable areas cover within each catchment. The smaller the difference between the catchment area and protected areas, the higher the vulnerability status of the catchment became.

Lastly, the ecosystem status of each catchment was extracted using the SANBI data set that classifies areas according to whether an ecosystem is critically threatened, threatened, vulnerable or least threatened. This was also done using a spatial join; however, here the attribute containing the status was joined to the catchments data set. These were the variables needed to identify the catchments that would potentially be most vulnerable if contaminants were found. With the information created in the catchment database and using the “Select by” attribute function, it was now possible to select the catchment areas with high population density, high number of potential containment outlets, and a larger number of vulnerable areas that can be affected.

Using Structured Query Language (SQL) within ArcGIS software using the “Select by” attribute tool, the most vulnerable catchments were extracted. This was done in phases within one SQL query. Phase 1 was to identify the population density within Gauteng per catchment. If the population density was more than a third of the total population, it was added to the query. Phase 2 was to identify the summed number of medical facilities within Gauteng. If a catchment had more than a third of the medical facilities, it was added to the query. More than a third was considered as it was the average mode per catchment. If a catchment had more than two WWTPs, it was also added to the query. This parameter was based on the average number of WWTPs located within each catchment. This formed part of the Phase 3. Phase 4 extracted catchments that had a critically threated ecosystem status from the attribute table within the database. If the area of protected areas for a catchment was more than 1000 ha, it was added to the query for selection. The catchment(s) had to fulfil all the above requirements for it to be selected as a vulnerable catchment. Furthermore, the river data set was overlayed with Gauteng and the river sections that overlapped the vulnerable areas were extracted using a select by location tool.

3.5.2.4 Selecting vulnerable catchment(s) (agricultural chemicals)

To determine the vulnerable catchments due to ECPs from agricultural holdings (areas where the land use was indicated as agricultural), the first step was to locate all the agricultural holdings within Gauteng bases using data from the Department of Agriculture Forestry and Fisheries. This was completed by importing the shapefile into ArcGIS, adding new fields to the attribute table, and using a calculate geometry tool within the attribute table to calculate the areas of the individual holdings. The summed area of the agricultural holdings per catchment were then calculated by first joining the agricultural holding layer with the Gauteng catchment by location, and then applying a join via attribute to the original data set where all the information of the variables was stored. Furthermore, using SQL, as with the first selection done above, the “select by” attribute tool was used to compute the most vulnerable catchments regarding agricultural holdings instead of medical facilities and WWTPs. Once again, this was done in phases within one SQL query.

Phase 1 was to identify the population density within Gauteng per catchment that fell in the highest category within the natural break selection and it was added to the query. Phase 2 was to extract all the catchments that had an endangered ecosystem status from the attribute table within the database. Phase 3 was to select protected areas where the area for each catchment was more than 1000 ha and add these to the query for selection. Lastly, the agricultural holdings that fell into the largest category (with their summed area being 7000 ha or more per catchment) were added to the query. The size limits were obtained based on a visual inspection of the data, based on what was perceived to be a significantly large area compared to the total for the province. The SQL query was based on “AND” functions, and only the catchment(s) that fulfilled all the above conditions were selected and stated as vulnerable catchment(s). Furthermore, the river data set was again overlayed with Gauteng and the river sections that overlapped the vulnerable areas were extracted using a select by location tool.

3.5.2.5 Displaying results

To display the outputs, the layers were scaled, categorised and symbolised. Each of the variables and some orientation data was employed to create maps to depict each stage of the methodology together with the final results displaying the vulnerable catchments and river sections for both the anthropogenic and agricultural holdings.

3.6 Results

3.6.1 Results obtained by the prioritisation approach

Table 3 contains the results from the hazard-based prioritisation of the 168 ECPs analysed in this study. The rank that each ECP attained for each analysed category (partition coefficient, toxicity and half-life in water) is included within the table. The total score for each respective ECP was calculated by summing together the aforementioned ranks. These scores were then used to give each individual ECP its final ranking. The values in the final ranking column of the table is thus representative of the final rank of each individual ECP in terms of the potential hazard it poses following a PBT approach.

Table 3: Results of preliminary prioritisation of ECPs arranged alphabetically per compound class. The source of data was Sigma-Aldrich MSDSs unless otherwise indicated

Substance	CAS No.	Partition Coefficient		Toxicity		Degradation Rates		Total Score	Final Ranking
		Value	Partition Coefficient Rank	Acute Toxicity Rat (LD50 Oral in mg·kg^{-1})	Toxicity Rank	Half-life in Water (days) (www.pbtprofiler.net)	Half-life Rank		
Pharmaceuticals: Analgesics and anti-inflammatory drugs									
Acetaminophen	103-90-2	log P: 0.31 (Hansch et al., 1995)	86	1 944	91	15	5	182	88
Acetylsalicylic acid	50-78-2	log Pow: 1.19	75	1 500	81	15	5	161	77
Antipyrine	60-80-0	log Pow: 0.538	83	1 705	86	15	5	174	83
Benzocaine	94-09-7	XLogP3 1.9 (Pubchem)	63	3042	103	15	5	171	82
Codeine	76-57-3	XLogP3 1.1 (Pubchem)	77	427	44	60	3	124	55
Dextropropoxyphene	1639-60-7	log Kow: 4.18	31	84	17	38	4	52	13
Diclofenac	15307-86-5	XLogP3 4.4 (Pubchem)	28	62.5	15	38	4	47	11
Fenoprofen	34597-40-5	XLogP3 3.3 (Pubchem)	42	> 200	28	15	5	75	20
Ibuprofen	15687-27-1	log Kow 2.48 (Scheytt et al., 2005)	56	636	50	15	5	111	43
Indomethacin	53-86-1	XLogP3 4.3 (Pubchem)	29	12	5	38	4	38	7
Ketoprofen	22071-15-4	log Pow 0.97 (Drugs.com)	78	62.4	14	15	5	97	34

Substance	CAS No.	Partition Coefficient		Toxicity		Degradation Rates		Total Score	Final Ranking
		Value	Partition Coefficient Rank	Acute Toxicity Rat (LD50 Oral in mg·kg^{-1})	Toxicity Rank	Half-life in Water (days) (www.pbtprofiler.net)	Half-life Rank		
Ketorolac	66635-83-4	XLogP3 1.9 (Pubchem)	63	189	27	38	4	94	31
Mefenamic acid	61-68-7	log Pow 5.33 (chemspider.com)	15	740	57	38	4	76	21
Naproxen	22204-53-1	XLogP3 3.3 (Pubchem)	42	248	32	15	5	79	23
Propyphenazone	479-92-5	XLogP3 1.7 (Pubchem)	65	860	63	38	4	132	59
Salicylic acid	69-72-7	log Pow: 2.21	58	891	65	15	5	128	57
Pharmaceuticals: Antibiotics									
4-aminoantipyrine [4-AA]	83-07-8	XLogP3 0.1 (Pubchem)	90	1700	85	15	5	180	87
Amoxicillin	26787-78-0	XLogP3 -2 (Pubchem)	106	> 15 000	127	38	4	237	111
Azithromycin	83905-01-5	XLogP3 4 (Pubchem)	33	> 2 000	93	180	1	127	56
Cefazolin	25953-19-9	XLogP3 -0.4 (Pubchem)	95	> 11 000	123	38	4	222	107
Cefotaxime	63527-52-6	XLogP3 -1.4 (Pubchem)	103	> 20 000	130	38	4	237	111
Chlortetracycline	57-62-5	log Pow: -0.62	98	3 000	102	180	1	201	99
Ciprofloxacin	85721-33-1	XLogP3 -1.1 (Pubchem)	102	> 2 000	93	60	3	198	96

Substance	CAS No.	Partition Coefficient		Toxicity		Degradation Rates		Total Score	Final Ranking
		Value	Partition Coefficient Rank	Acute Toxicity Rat (LD50 Oral in $\text{mg}\cdot\text{kg}^{-1}$)	Toxicity Rank	Half-life in Water (days) (www.pbtprofiler.net)	Half-life Rank		
Clarithromycin	81103-11-9	XLogP3 3.2 (Pubchem)	43	1 270	76	180	1	120	51
Doxycycline	564-25-0	XLogP3 -0.7 (Pubchem)	99	262	35	60	3	137	62
Enrofloxacin	93106-60-6	XLogP3 -0.2 (Pubchem)	94	5 000	113	180	1	208	103
Erythromycin	114-07-8	XLogP3 2.7 (Pubchem)	52	4 600	111	180	1	164	79
Fluconazole	86386-73-4	log Pow: 1.473	70	1 271	77	180	1	148	70
Lincomycin	154-21-2	XLogP3 0.2 (Pubchem)	88	262	35	38	4	127	56
Metronidazole	443-48-1	XLogP3 0 (Pubchem)	91	3 000	102	38	4	197	95
Nalidixic acid	389-08-2	log Pow: 1.41	72	1 160	74	38	4	150	72
Norfloxacin	70458-96-7	XLogP3 -1 (Pubchem)	101	> 4 000	109	60	3	213	105
Ofloxacin	82419-36-1	XLogP3 -0.4 (Pubchem)	96	3 590	107	180	1	204	102
Oxytetracycline	79-57-2	XLogP3 -1.6 (Pubchem)	104	3 000	102	60	3	209	104
Roxithromycin	80214-83-1	XLogP3 3.1 (Pubchem)	45	830	62	180	1	108	41
Sulfamethazine	57-68-1	XLogP3 0.3 (Pubchem)	87	50 000	133	38	4	224	109

Substance	CAS No.	Partition Coefficient		Toxicity		Degradation Rates		Total Score	Final Ranking
		Value	Partition Coefficient Rank	Acute Toxicity Rat (LD50 Oral in mg·kg^{-1})	Toxicity Rank	Half-life in Water (days) (www.pbtprofiler.net)	Half-life Rank		
Sulfapyridine	144-83-2	XLogP3 0 (Pubchem)	91	15 800	128	38	4	223	108
Sulfisomidine	208-204-3	XLogP3 1.2 (Pubchem)	74	3 000	102	38	4	180	87
Sulfamethoxazole	723-46-6	XLogP3 0.9 (Pubchem)	80	6 200	116	38	4	200	98
Tetracycline	60-54-8	XLogP3 -2 (Pubchem)	106	807	60	60	3	169	80
Trimethoprim	738-70-5	XLogP3 0.9 (Pubchem)	80	> 5 300	114	60	3	197	95
Pharmaceuticals: Beta blockers									
Acebutolol	34381-68-5	XLogP3 1.7 (Pubchem)	65	3 000	102	38	4	171	82
Atenolol	29122-68-7	XLogP3 0.2 (Pubchem)	88	> 2 000	93	38	4	185	91
Celiprolol	260-497-7	XLogP3 1.9 (Pubchem)	93	> 2 157	94	38	4	161	77
Metoprolol	37350-58-6	XLogP3 1.9 (Pubchem)	63	5 500	115	38	4	182	88
Pindolol	13523-86-9	XLogP3 1.8 (Pubchem)	64	263	36	15	5	105	39
Propranolol	525-66-6	XLogP3 3 (Pubchem)	46	660	53	15	5	104	38
Sotalol	3930-20-9	XLogP3 0.2 (Pubchem)	88	3 450	106	15	5	199	97

Substance	**CAS No.**	**Partition Coefficient**		**Toxicity**		**Degradation Rates**		**Total Score**	**Final Ranking**
		Value	**Partition Coefficient Rank**	**Acute Toxicity Rat (LD50 Oral in mg·kg^{-1})**	**Toxicity Rank**	**Half-life in Water (days) (www.pbtprofiler.net)**	**Half-life Rank**		
Pharmaceuticals: Blood lipid lowering agents									
Bezafibrate	41859-67-0	XLogP3 3.8 (Pubchem)	37	1 082	72	60	3	112	44
Fenofibric acid	42017-89-0	log Pow: 3.895	35	1 242	75	38	4	114	46
Gemfibrozil	25812-30-0	XLogP3 3.8 (Pubchem)	37	1 414	80	38	4	121	52
Mevastatin	73573-88-3	XLogP3 3.9 (Pubchem)	34	> 2 000	93	15	5	132	59
Pravastatin	81093-37-0	XLogP3 1.6 (Pubchem)	67	12 000	124	15	5	196	94
Pharmaceuticals: Neuroactive compounds									
Carbamazepine	298-46-4	log Kow 1.51 (Scheytt et al., 2005)	68	1957	92	38	4	164	79
Citalopram	59729-33-8	XLogP3 3.2 (Pubchem)	43	825	61	180	1	105	39
Diazepam	439-14-5	XLogP3 3 (Pubchem)	48	249	33	38	4	83	26
Fluoxetine	54910-89-3	XLogP3 4 (Pubchem)	33	825	61	60	3	97	34
Imipramine	50-49-7	XLogP3 4.8 (Pubchem)	23	355	39	60	3	65	16
Paroxetine	61869-08-7	XLogP3 3.5 (Pubchem)	40	> 500	46	60	3	89	28

Substance	CAS No.	Partition Coefficient		Toxicity		Degradation Rates		Total Score	Final Ranking
		Value	Partition Coefficient Rank	Acute Toxicity Rat (LD50 Oral in mg·kg^{-1})	Toxicity Rank	Half-life in Water (days) (www.pbtprofiler.net)	Half-life Rank		
Temazepam	846-50-4	XLogP3 2.2 (Pubchem)	59	2000	93	38	4	156	74
Venlafaxine	93413-69-5	XLogP3 2.9 (Pubchem)	47	9 000	120	60	3	170	81
Contrast media									
Diatrizoic acid	117-96-4	XLogP3 1.8 (Pubchem)	64	12 300	125	180	1	190	92
Iohexol	66108-95-0	XLogP3 -3 (Pubchem)	109	20 000	130	60	3	242	113
Iomeprol		XLogP3 -2.3 (Pubchem)	108	14 300	126	60	3	237	111
Iopromide	73334-07-3	XLogP3 -2.1 (Pubchem)	107	21 403	131	60	3	241	112
PCPs: Cosmetics									
Galaxolide	1222-05-5	XLogP3 4.8 (Pubchem)	23	> 5 000	113	60	3	139	64
Tonalid	21145-77-7	XLogP3 5.3 (Pubchem)	16	570	47	60	3	66	17
Pharmaceuticals: Diuretics									
Furosemide	54-31-9	XLogP3 2 (Pubchem)	61	2 600	98	60	3	162	78
Hydrochlorothiazide	58-93-5	XLogP3 -0.1 (Pubchem)	93	> 10 000	121	60	3	217	106

Substance	CAS No.	Partition Coefficient		Toxicity		Degradation Rates		Total Score	Final Ranking
		Value	Partition Coefficient Rank	Acute Toxicity Rat (LD50 Oral in mg·kg^{-1})	Toxicity Rank	Half-life in Water (days) (www.pbtprofiler.net)	Half-life Rank		
Pharmaceuticals: Psycho-stimulants									
Caffeine	58-08-2	log Pow: -0.091 at 23°C	92	367.7	40	15	5	137	62
PCPs: Disinfectants									
Chloroform	67-66-3	log Pow: 1.97	62	908	68	38	4	134	60
Bromoacetic acid	79-08-3	log Pow: 0.41	85	50	11	8.7	6	102	37
Bromodichloromethane	75-27-4	XLogP3 2.4 (Pubchem)	57	1 388	79	75	2	138	63
Chloroacetonitrile	107-14-2	log Pow: 0.45	84	220	29	15	5	118	50
Dibromoacetic acid	631-64-1	XLogP3 1.5 (Pubchem)	69	1 737	87	15	5	161	77
Dibromoacetonitrile	3252-43-5	XLogP3 1.7 (Pubchem)	65	245	31	38	4	100	36
Dibromochloromethane	124-48-1	XLogP3 2.6 (Pubchem)	53	370	41	38	4	98	35
Dichloroacetic acid	79-43-6	log Pow: 0,942	79	2 820	100	15	5	184	90
Dichloroacetonitrile	3018-12-0	XLogP3 1.3 (Pubchem)	73	330	38	38	4	115	47
Formaldehyde	50-00-0	XLogP3 1.2 (Pubchem)	74	800	59	15	5	138	63
Monochloroacetic acid	79-11-8	log Pow: 0.2	88	90.4	20	15	5	113	45
Nitrosodimethylamine	62-75-9	XLogP3 -0.6 (Pubchem)	97	37	8	38	4	109	42

Substance	CAS No.	Partition Coefficient		Toxicity		Degradation Rates		Total Score	Final Ranking
		Value	Partition Coefficient Rank	Acute Toxicity Rat (LD50 Oral in mg·kg^{-1})	Toxicity Rank	Half-life in Water (days) (www.pbtprofiler.net)	Half-life Rank		
Nonylphenol	84852-15-3	log Pow: 5.4 at 23°C	14	580	48	38	4	66	17
Trichloroacetic acid	76-03-9	log Pow: 1.645	66	3 320	105	38	4	175	84
Trichloroacetonitrile	545-06-2	XLogP3 2.1 (Pubchem)	60	250	34	60	3	97	34
Triclosan	3380-34-5	log Pow: 4.7	24	3 700	108	60	3	135	61
Phthalates									
Benzyl butyl phthalate	85-68-7	log Pow: 4.91 at 20°C	20	2 330	97	15	5	122	53
Bis[2-ethylhexyl] phthalate	117-81-7	XLogP3 7.4 (Pubchem)	3	30 000	132	15	5	140	65
Bisphenol A	80-05-7	log Pow: 3.4 at 21.5°C	41	2000	93	38	4	138	63
Dibutyl phthalate	84-74-2	XLogP3 4.7 (Pubchem)	24	8 000	117	8.7	6	147	69
Diethyl phthalate	84-66-2	log Pow: 2.2 at 41°C	59	8 600	119	15	5	183	89
Diisobutyl phthalate	84-69-5	log Pow: 4.11 at 20°C	32	10 392	122	15	5	159	76
Dimethyl phthalate	131-11-3	log Pow: 1.47	71	8 200	118	15	5	194	93

Substance	CAS No.	Partition Coefficient		Toxicity		Degradation Rates		Total Score	Final Ranking
		Value	Partition Coefficient Rank	Acute Toxicity Rat (LD50 Oral in mg·kg^{-1})	Toxicity Rank	Half-life in Water (days) (www.pbtprofiler.net)	Half-life Rank		
Pesticides									
2,4-Dichlorophenoxyacetic acid [2,4-D]	94-75-7	log Pow: 2.81	50	375	42	38	4	96	33
2-methyl-4-chlorophenoxyacetic acid	94-74-6	XLogP3 2.6 (Pubchem)	53	700	55	15	5	113	45
Acetochlor	34256-82-1	log Pow: 2.719	51	763	58	60	3	112	44
Aldicarb	116-06-3	XLogP3 1.1 (Pubchem)	77	0.5	1	38	4	82	25
Aldrin	309-00-2	log Pow: 6.50	9	39	9	180	1	19	2
Atrazine	1912-24-9	log Pow: 2,61 log Pow: 5	19	672	54	60	3	76	21
Chlorpyrifos	2921-88-2	log Pow: 5.27	17	82	16	180	1	34	6
Clofibrin acid/Clofibric acid	882-09-7	log Kow 2.88 (Scheytt et al., 2005)	48	897	66	38	4	118	50
Cyanazine	21725-46-2	XLogP3 2.2 (Pubchem)	59	149	24	180	1	84	27
Cypermethrin	52315-07-8	log Pow: 5	19	57.5	13	180	1	33	5
Dichlorodiphenyldichloro-ethane	72-54-8	log Pow: 6.02	11	113	21	180	1	33	5
DDE	72-55-9	log Pow: 6.51	8	880	64	180	1	73	19
DDT	50-29-3	log Pow: 6.91	5	87	18	180	1	24	4
Deltamethrin	52918-63-5	XLogP3 6.2 (Pubchem)	10	9.36	4	60	3	17	1

Substance	CAS No.	Partition Coefficient		Toxicity		Degradation Rates		Total Score	Final Ranking
		Value	Partition Coefficient Rank	Acute Toxicity Rat (LD50 Oral in mg·kg^{-1})	Toxicity Rank	Half-life in Water (days) (www.pbtprofiler.net)	Half-life Rank		
Dieldrin	60-57-1	XLogP3 3.7 (Pubchem)	38	37	8	180	1	47	11
Diquat dibromide	85-00-7	log Kow: 4.60	25	120	22	38	4	51	12
Endosulphan	115-29-7	log Pow: 3.83	36	18	7	180	1	44	10
Endosulphan sulphate	1031-07-8	log Pow: 3.66	39	8	3	180	1	43	9
Endrin	72-20-8	log Pow: 5.20	18	3	2	180	1	21	3
Enilconazole	35554-44-0	XLogP3 3.8 (Pubchem)	37	227	30	60	3	70	18
Fenoprop	93-72-1	XLogP3 3.8 (Pubchem)	37	650	52	38	4	93	30
Glyphosate	1071-83-6	XLogP3 -4.6 (Pubchem)	110	4 873	112	15	5	227	110
Heptachlor epoxide	1024-57-3	log Pow: 5.40	14	15	6	180	1	21	3
Heptachlor	76-44-8	XLogP3 4.3 (Pubchem)	29	40	10	180	1	40	8
Hexachlorobenzene [HCB]	118-74-1	XLogP3 5.7 (Pubchem)	13	10 000	121	180	1	135	61
Hexachlorocyclohexane isomers	319-84-6	log Pow: 3.80	37	177	26	180	1	64	15
Hexazinone	51235-04-2	XLogP3 1.3 (Pubchem)	73	1 690	84	38	4	161	77
Imidacloprid	138261-41-3	XLogP3 0.8 (Pubchem)	81	410	43	60	3	127	56
Lindane	58-89-9	Pow: 3.5 at 22°C	40	88	19	180	1	60	14

Substance	CAS No.	Partition Coefficient		Toxicity		Degradation Rates		Total Score	Final Ranking
		Value	Partition Coefficient Rank	Acute Toxicity Rat (LD50 Oral in mg·kg^{-1})	Toxicity Rank	Half-life in Water (days) (www.pbtprofiler.net)	Half-life Rank		
Metazachlor	67129-08-2	log Pow: 2.49	55	1000	70	60	3	128	57
Methoxychlor	67129-08-2	log Pow: 2.49	55	1 855	90	60	3	148	71
Metolachlor	51218-45-2	XLogP3 3.1 (Pubchem)	45	2 200	95	60	3	143	68
Paraquat	1910-42-5	XLogP3 1.7 (Pubchem)	65	57	12	38	4	81	24
Simazine	122-34-9	XLogP3 2.2 (Pubchem)	59	971	69	60	3	121	58
Tebuthiuron	34014-18-1	XLogP3 1.6 (Pubchem)	67	644	51	38	4	122	53
Vinclozolin	50471-44-8	XLogP3 3.1 (Pubchem)	45	10 000	121	60	3	169	80
PAHs									
Acenaphthene	83-32-9	log Pow: 3.9	34	1 700	85	38	4	123	54
Acenaphthylene	208-96-8	XLogP3 3.7 (Pubchem)	38	1 760	88	15	5	131	58
Benzo[a]pyrene	50-32-8	log Pow: 5.97	12	1 600	82	60	3	97	34
Fluoranthene	206-44-0	log Kow 4.90 (4.58) Ministry of Environment, Lands and Parks Province of British Columbia	21	2000	93	60	3	117	49

Substance	CAS No.	Partition Coefficient		Toxicity		Degradation Rates		Total Score	Final Ranking
		Value	Partition Coefficient Rank	Acute Toxicity Rat (LD50 Oral in mg·kg^{-1})	Toxicity Rank	Half-life in Water (days) (www.pbtprofiler.net)	Half-life Rank		
Fluorene	86-73-7	XLogP3 4.2 (Pubchem)	30	> 2 000	93	15	5	128	57
Naphthalene	91-20-3	log Pow: 3.30	42	490	45	38	4	91	29
Phenanthrene	85-01-8	log Pow: 4.57	26	1 800	89	60	3	118	50
Pyrene	129-00-0	log Pow: 4.88	22	2 700	99	60	3	124	55
Pharmaceuticals: ARVs									
Abacavir	136470-78-5	XLogP3 0.9 (Pubchem)	80	2000	93	38	4	177	85
Lamivudine	134678-17-4	log Pow: -0.93	100	2000	93	15	5	198	96
Stavudine	3056-17-5	log Kow: 0.144	89	4 000	109	15	5	203	101
Zidovudine	30516-87-1	XLogP3 0 (Pubchem)	91	3 084	104	15	5	200	98
Pharmaceuticals: Antivirals									
Famciclovir	104227-87-4	log Pow: 0,739	82	2000	93	38	4	179	86
Penciclovir	39809-25-1	XLogP3 -1.6 (Pubchem)	104	2000	93	15	5	202	100
Ribavirin	36791-04-5	XLogP3 -1.8 (Pubchem)	105	2 700	99	15	5	209	104
Additional Substances									
Benzophenone	119-61-9	log Pow: 3.18	44	> 10 000	121	15	5	170	81
Cinchonidine	485-71-2	XLogP3 2.7 (Pubchem)	52	316	37	38	4	93	30

Substance	CAS No.	Partition Coefficient		Toxicity		Degradation Rates		Total Score	Final Ranking
		Value	Partition Coefficient Rank	Acute Toxicity Rat (LD50 Oral in mg·kg^{-1})	Toxicity Rank	Half-life in Water (days) (www.pbtprofiler.net)	Half-life Rank		
Cinchonine	118-10-5	XLogP3 2.7 (Pubchem)	52	152	25	38	4	81	24
Clofibric acid	882-09-7	XLogP3 2.6 (Pubchem)	53	897	67	38	4	124	55
Clotrimazole	23593-75-1	XLogP3 5 (Pubchem)	19	708	56	60	3	78	22
Diphenylamine	122-39-4	log Pow: 3.5	40	1 120	73	38	4	117	49
Diuron	330-54-1	log Pow: 2.84 at 20°C	49	1 017	71	38	4	124	55
Ephedrine	299-42-3	XLogP3 0.9 (Pubchem)	80	600	49	15	5	134	60
Ethinylestradiol	57-63-6	XLogP3 3.7 (Pubchem)	38	2 952	101	60	3	142	67
Ifosfamide	3778-73-2	XLogP3 0.9 (Pubchem)	80	143	23	38	4	107	40
Minoxidil	38304-91-5	XLogP3 1.2 (Pubchem)	74	1 321	78	60	3	155	73
Nicotine	54-11-5	log Pow: 1.17	76	50	11	38	4	91	29
Omeprazole	73590-58-6	XLogP3 2.2 (Pubchem)	59	2 210	96	60	3	158	75
Phenytoin	57-41-0	XLogP3 2.5 (Pubchem)	54	1 635	83	38	4	141	66
Ranitidine	66357-35-5	XLogP3 0.3 (Pubchem)	87	> 5 000	113	38	4	204	102

Substance	**CAS No.**	**Partition Coefficient**		**Toxicity**		**Degradation Rates**		**Total Score**	**Final Ranking**
		Value	**Partition Coefficient Rank**	**Acute Toxicity Rat (LD50 Oral in mg·kg^{-1})**	**Toxicity Rank**	**Half-life in Water (days) (www.pbtprofiler.net)**	**Half-life Rank**		
Tamoxifen	10540-29-1	XLogP3 7.1 (Pubchem)	4	4 100	110	60	3	117	49
Telmisartan	144701-48-4	XLogP3 6.9 (Pubchem)	6	> 2 000	93	60	3	102	37
				BFRs					
Decabromodiphenyl ether	1163-19-5	XLogP3 10.4 (Pubchem)	1	2 000	93	180	1	95	32
Hexabromocyclododecane	3194-55-6	XLogP3 7.1 (Pubchem)	4	10 000	121	60	3	128	57
Octabromodiphenyl ether	32536-52-0	XLogP3 9 (Pubchem)	2	5 000	113	180	1	116	48
Pentabromodiphenyl ether	32534-81-9	XLogP3 6.9 (Pubchem)	6	5 000	113	180	1	120	51
Tetrabromobisphenol A	79-94-7	XLogP3 6.8 (Pubchem)	7	5 000	113	180	1	121	52

3.6.2 Results obtained by the GIS (potential sampling site selection process)

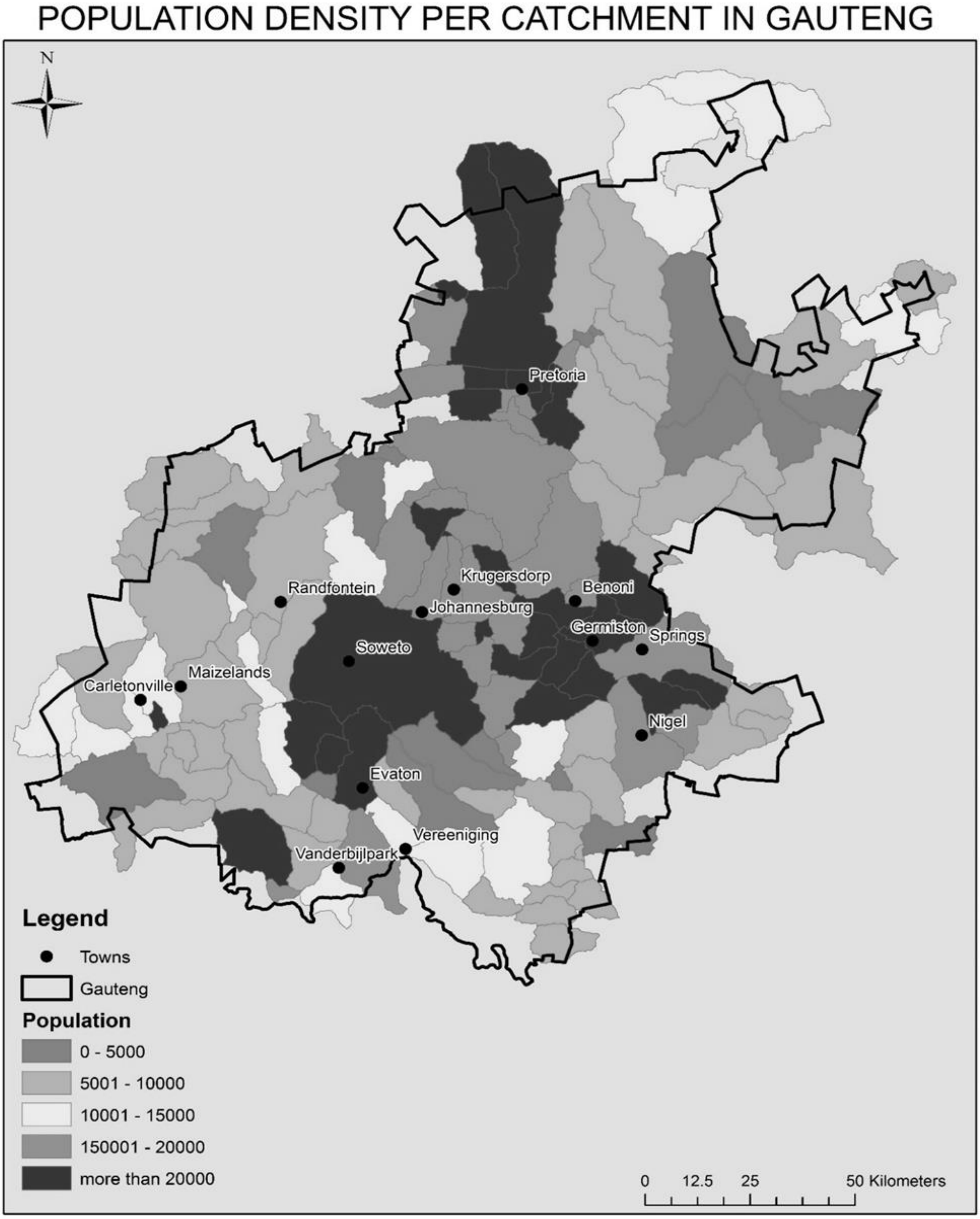

Figure 4: Population density per catchment in Gauteng

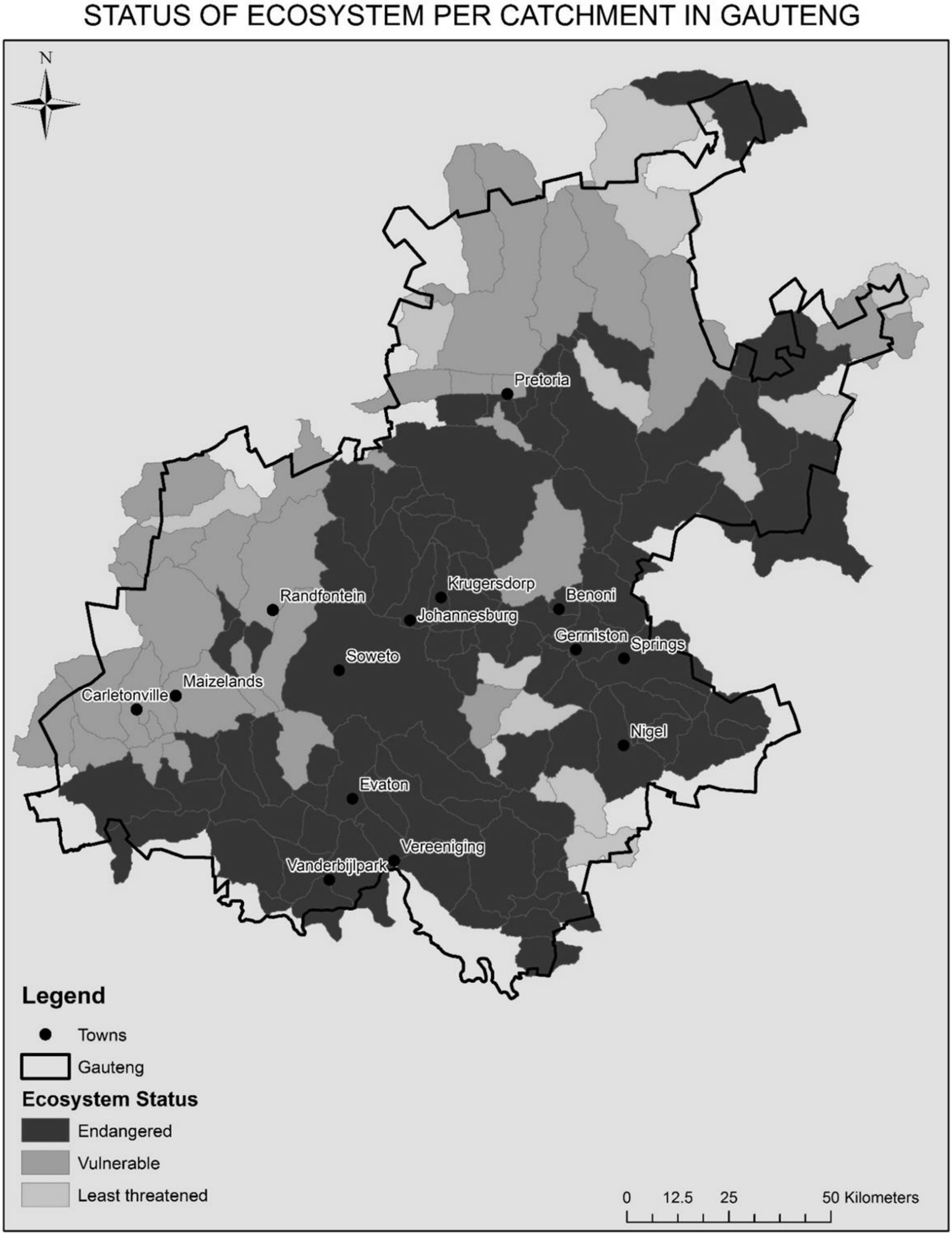

Figure 5: Status of ecosystem per catchment in Gauteng

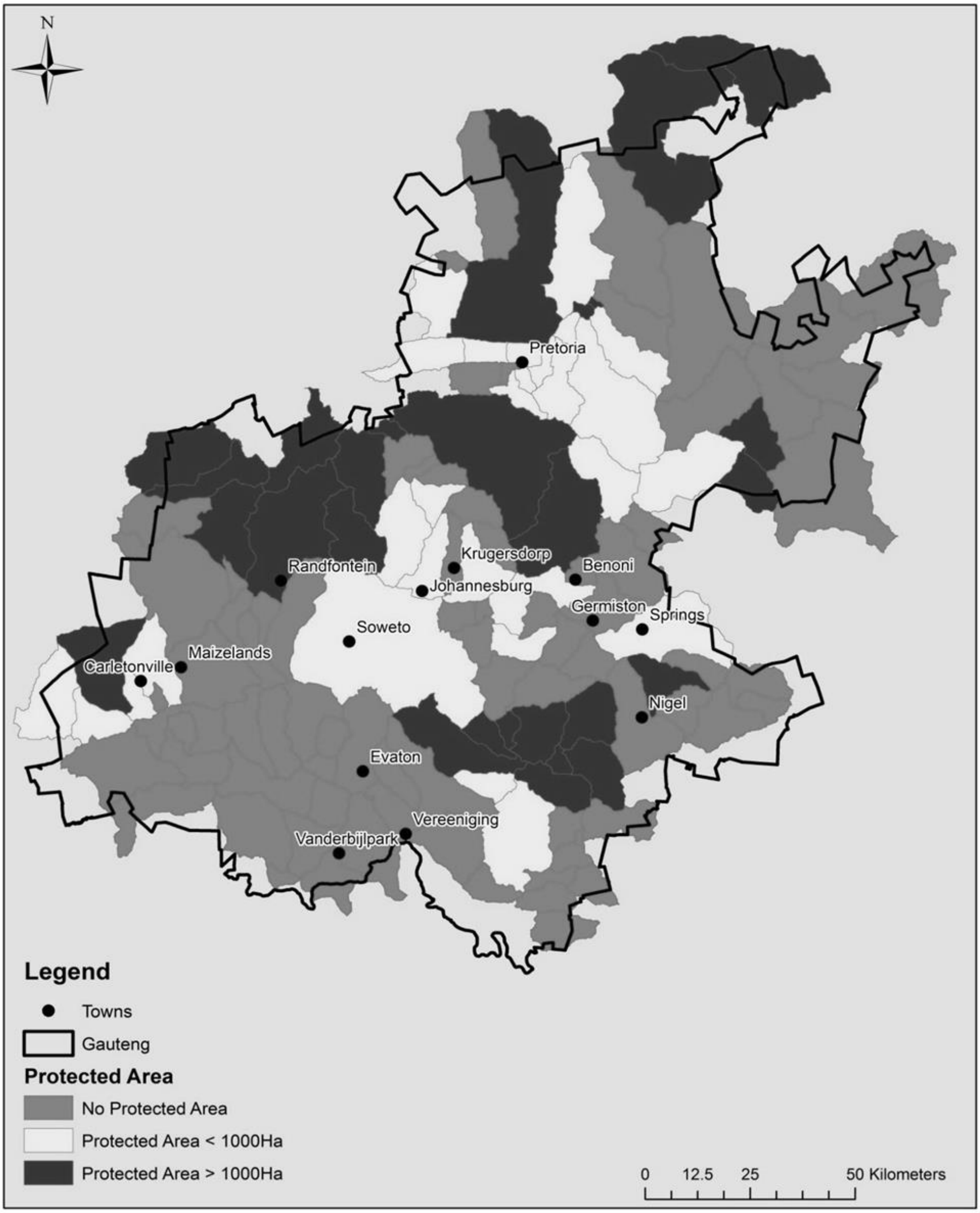

Figure 6: Protected areas per catchment in Gauteng

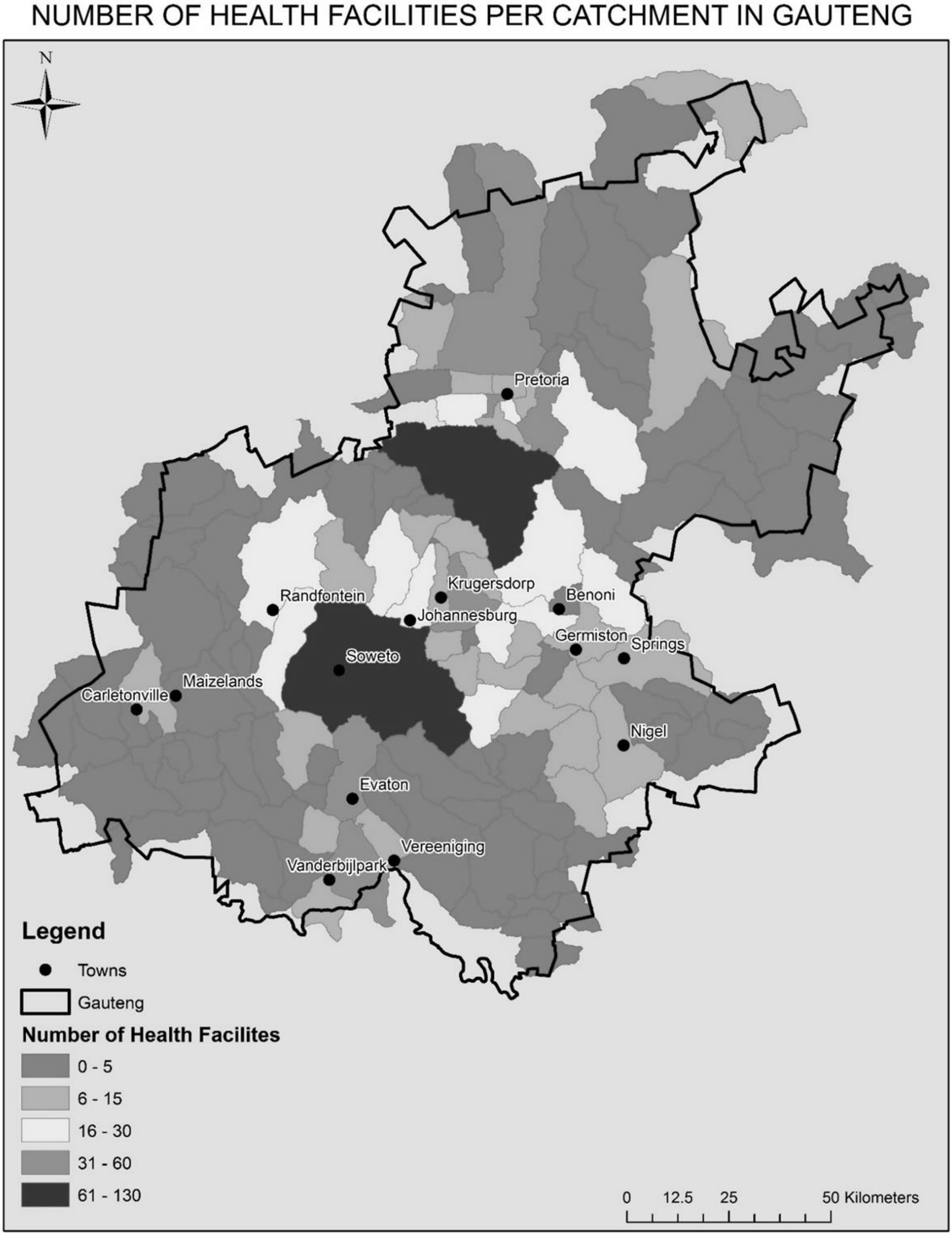

Figure 7: Number of health facilities per catchment in Gauteng

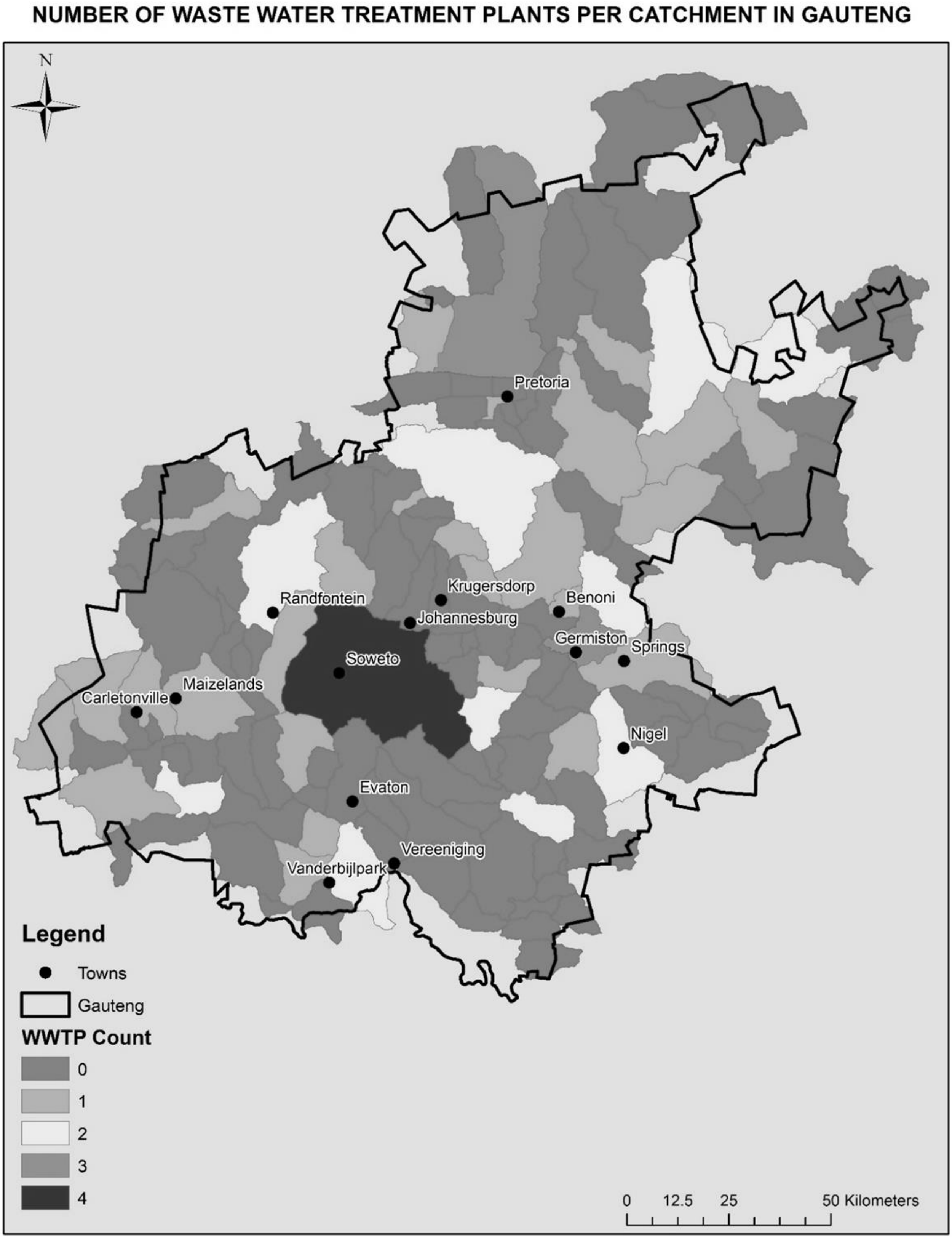

Figure 8: Number of WWTPs per catchment in Gauteng

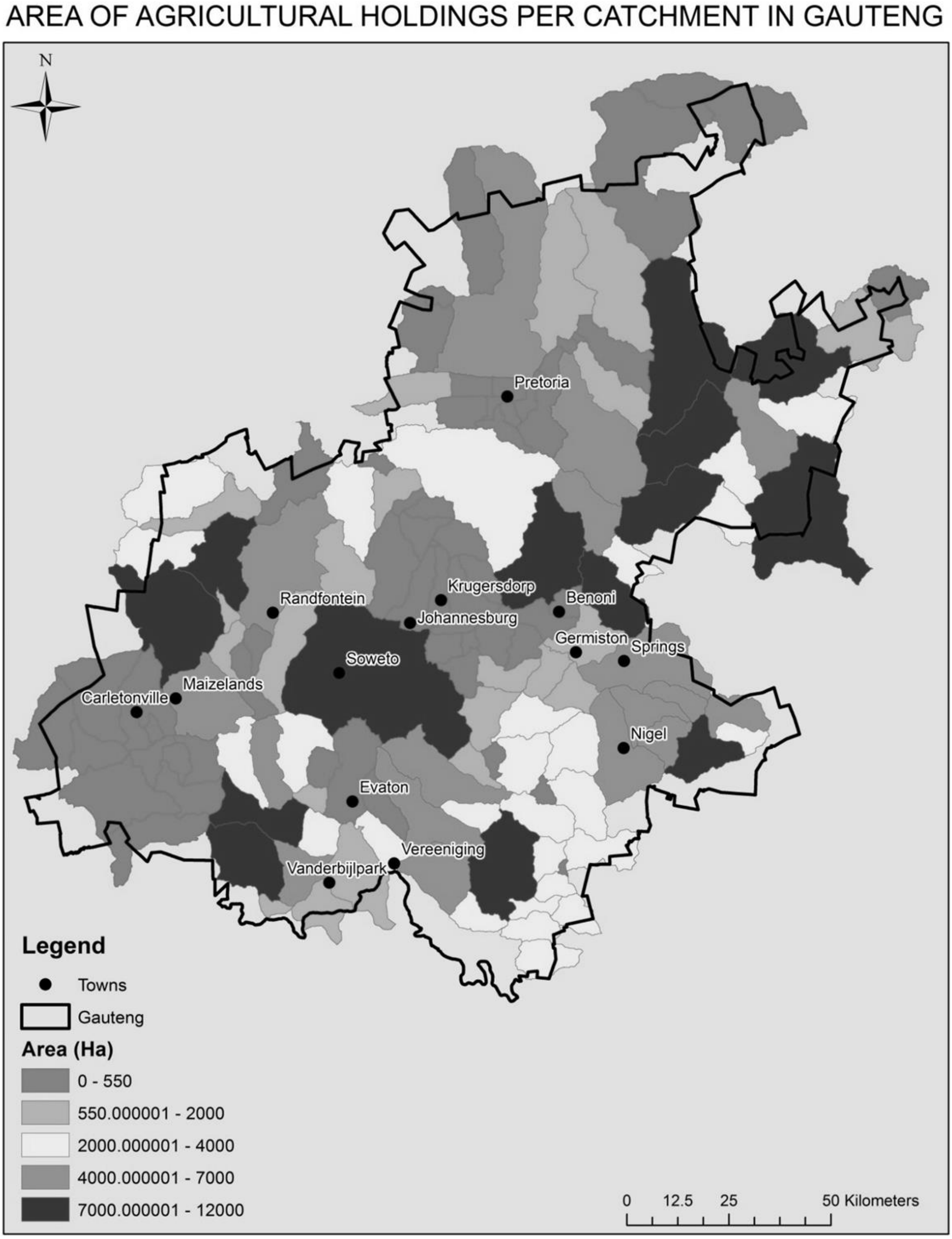

Figure 9: Area of agriculture per catchment in Gauteng

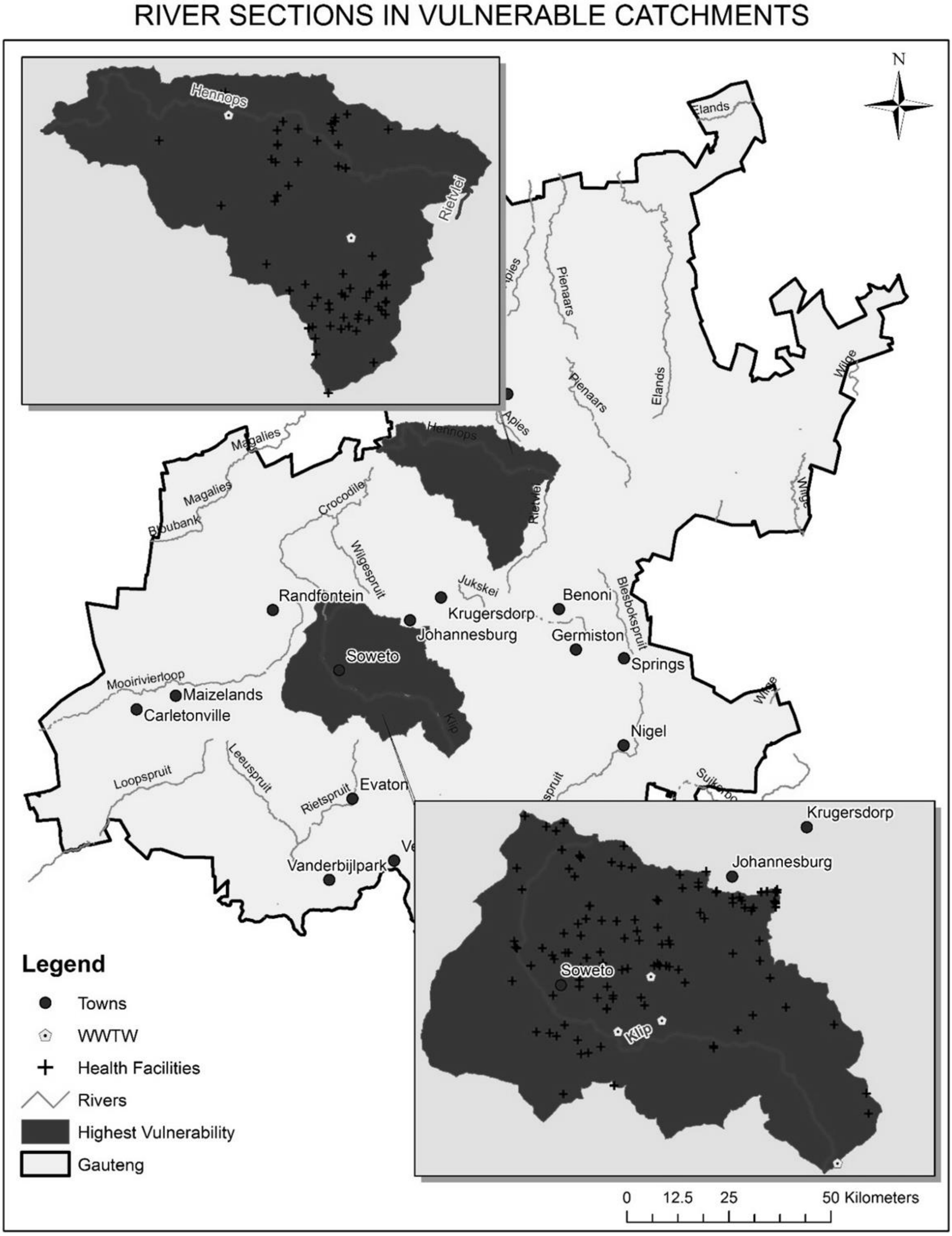

Figure 10: River sections in most vulnerable catchments for anthropogenic substances

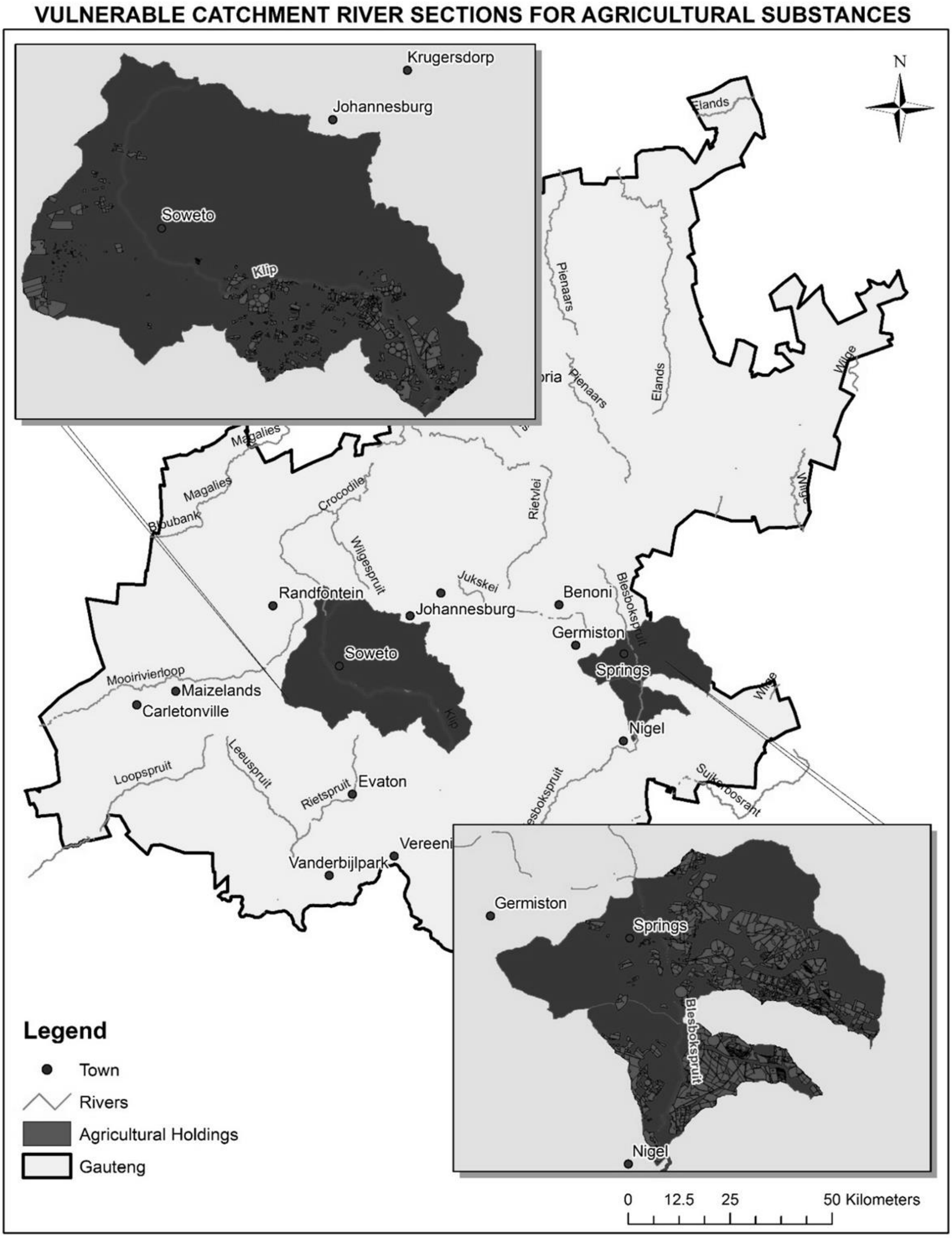

Figure 11: Vulnerable catchment river sections for agricultural substances

3.7 Discussion

3.7.1 Discussion of the results obtained by the prioritisation approach

Gauteng is a highly urbanized province and one of the most densely populated areas in South Africa. Considering this, the population density distribution per catchment in Gauteng will be higher in more urbanized areas, as is illustrated by the map in Figure 4 where catchments close to major towns have higher population density than those on the outskirts of Gauteng. The number of health facilities was proportional to the population density: the more populated the catchment, the more health facilities were found. Furthermore, looking at the overall ecosystem status in Gauteng, it can be noted that more than 80% of all catchments within Gauteng have an endangered status. This means that more than 60% of the ecosystem has been significantly degraded and has 60 or more threatened red data list plant species and irreversible loss of natural habitat. This status is based on criteria developed by the Department of Environmental Affairs in the National Environmental Management: Biodiversity Act No. 10 of 2004 (DEA, 2004).

From Table 3 it appears that the hazard posed by pharmaceuticals varies between subdivisions, with analgesics and anti-inflammatory drugs generally posing a greater hazard than the antibiotics. This statement is supported by the fact that 9 of the 16 chemicals (56.25%) that fall into the category of analgesics and anti-inflammatory drugs had a final hazard ranking of under 50 (lower ranks indicating the higher priority and potential hazard). Based on the total number of compounds considered, this puts these chemicals well into the upper 50% of the total ECPs analysed in terms of the potential hazard they pose. As a class of ECPs, analgesics and anti-inflammatory drugs definitely warrant further investigation and monitoring to determine occurrence data based on the findings of the hazard-based analysis conducted in this study.

Of the antibiotics, only one (Roxithromycin) of the 25 considered had a final hazard ranking that was lower than 50. This would initially lead to the conclusion that this group of chemicals poses relatively inconsequential harm to the environment; however, the capacity of antibiotics to increase the drug resistance of naturally occurring and potentially disease-causing microbes in the environment means that this class of ECPs might well pose significant potential harm to both the environment and human health. It is worth noting that the lack of published log K_{ow} values could also contribute to the erroneous conclusion of inconsequential risk. The hazard that antibiotics pose might just be less direct as well as less obvious upon initial observation and analysis.

From an international perspective, it would seem that the research focus surrounding the threat that antibiotics pose has shifted from looking for a direct threat for example toxicity to looking for secondary problems they could cause such as the increased resistance of pathogens in water resources (Kümmerer, 2009). This was likely the result of numerous studies reporting the small potential for direct risk to human health that is posed by antibiotics in water bodies (Kümmerer, 2009).

Two of the seven beta blockers analysed ranked in the upper 50% of the total chemicals. The remaining beta blockers had ranks of 77 and higher. This means that the hazards they pose would be largely insignificant when compared to most other analysed ECPs. This means that apart from pindolol and propranolol (ranked 39 and 38 respectively), beta blockers are not expected to cause major ecological damage when compared to other ECP classes.

Most blood lipid lowering class of ECPs had final ranks between 40 and 60 with pravastatin being the only exception as it had a rank of 94. Based on the rankings obtained by this class, further investigation into the risk that they pose should be conducted, especially into their ability to bioaccumulate as it is their partition coefficients that result in their intermediate ranks.

Neuroactive compounds were an ECP class found to be quite hazardous based on the final hazard-based ranking that these chemicals attained. Five of the eight neuroactive compounds that formed part of the analysis had final rankings of less than 50, and thus ranked well within the upper 50% of the ECPs that were analysed. Only carbamazepine, temazepam and venlafaxine ranked high enough to be considered non-threatening when compared to other ECPs based on this approach. Mention should be made of the capacity to elicit behavioural changes in organisms that came into contact with neuroactive compounds. This capacity did not form part of the analysis in the applied hazard-based prioritisation and the full hazard posed by neuroactive compounds might have been underestimated, especially from an environmental and ecological point of view. This statement is further supported by the fact that the chemicals can have impacts on the priority of aquatic organisms even at low concentrations (Rivetti et al., 2015). For a better understanding into the hazard that is posed by neuroactive compounds when they enter the environment, further investigations into their behavioural altering capabilities and the consequences thereof should be conducted. These results should then be incorporated into future hazard or risk-based analysis of both ECPs and neuroactive compounds specifically.

From the class of cosmetics, tonalid obtained a final ranking of 17, thus meaning it could pose harm to the environment and human health when compared to other ECPs. The other cosmetic that was analysed, namely galaxolide, had moderate potential as a hazard to the environment and human health based on its final rank of 64. The moderate risk rankings obtained by cosmetics in this study correlate well with other studies that focus on cosmetics in water resources, such as the study by Diamond et al. (2011). Within the review of emerging contaminants by Thomaidis et al. (2012), certain cosmetic products such as synthetic musk was mentioned due to its tendency to accumulate in sediment and certain organic matter; however, the low toxicity scores for this class of chemicals make them less harmful and thus of lower priority.

Similarly, the hazard and final rank that was obtained for caffeine was also moderate. Caffeine’s ability to cause harm could potentially be amplified by its widespread and frequent use. This could potentially also be the case with BFRs as these substances are used in numerous manufactured goods and their addition to many of these goods is required by law such as for example in building materials. The BFRs analysed obtained quite similar final rankings of between 32 and 57. This is the case largely due to the persistence and log K_{ow} scores of these compounds. As such, this class of compounds warrants further investigation and should be considered as a priority ECP due to the nearly unanimous elevated potential hazard that is posed by all of the chemicals in this class. BFRs have in recent years become the focus of numerous scientific inquiries, especially relating to their potential to cause harm to the environment both internationally (Segev et al., 2009) and locally (Olukunle et al., 2012). The motivation behind the analysis of these compounds correlates with the reasons for the ranks they obtained in this study as their persistence and log K_{ow} scores have been stated as the reasons why they pose a potential threat to the environment and need to be further investigated (Alaee et al., 2003).

Pesticides were the largest class of ECPs that were analysed in terms of sheer numbers (36 of a total of 168). As previously mentioned, the risk posed by pesticides is likely to be elevated in South Africa due to the size of the agricultural sector and consequently the high usage of pesticides. This class of ECPs featured some of the chemicals that obtained the lowest total hazard ranking and has thus the highest potential to cause harm to the environment and human health. This class contained chemicals that obtained total hazard rankings from 1 through to 6. Thus, the class contained the most hazardous chemicals of all the ECPs considered. Of the chemicals, 27 out of the 36 (75%) that constituted the ECP class of pesticides had final hazard rankings of 50 or lower, meaning that they are ranked well within the top 45% of ECPs regarding their potential to cause harm. The high ranks that pesticides achieved are caused by their long half-lives in conjunction with their high toxicity scores. These facts in conjunction with the aforementioned widespread and frequent use of pesticides in South Africa mean that this class of ECP will likely pose the greatest hazard to the environment and human health in South Africa out of all the existing classes of ECPs.

Pesticides are included in numerous international studies on ECPs, which include a notable study by Diamond et al. (2011). This study found that pesticides are one of the ECP classes of the highest concern in a European setting. This is largely caused by the physical and chemical characteristics of pesticides, their widespread use, and the sheer number that are produced and used. The diversity of this compound class was also reported to be noteworthy. An article by Thomaidis et al. (2012) supported these findings in their review of emerging contaminants. Other studies that included pesticides in their studies and reported similar findings include Haarhoff et al. (2015), Gavrilescu et al. (2014), Deblonde et al. (2011) and Bueno et al. (2012).

Due the lack of data in the categories of toxicity and aquatic half-life, not all the PAHs could be prioritised and compared with other ECPs. The only PAHs for which a full set of required data could be obtained and compared with other classes of ECPs were acenaphthene, acenaphthylene, benzo[a]pyrene, fluoranthene, prioriti, naphthalene, phenanthrene and finally pyrene. All these chemicals were ranked within the upper 50% of ECPs in terms of their potential to be hazardous. Full data will be required before the other PAHs can be compared with other classes of ECPs as their toxicity equivalency scores only allow for them to be compared with each other and not with the rest of the ECPs. Based on the chemicals that could be analysed, this class should be considered a high priority class of ECPs. Therefore, the continued monitoring and studies to make full data available are required to assess the potential risk more accurately that they pose.

Disinfectants are a class of ECP that generally pose a great variety of hazards to both human health and the environment when compared with other ECPs. The hazards posed by this class can vary from substantial to essentially inconsequential. This is because the chemicals that constitute this class of ECP had final hazard rankings that fell between 17 (nonylphenol) and 90 (dichloroacetic acid). Thus, there is no universal statement that can be made regarding the hazard that is posed by this class in general. Chemicals of this class should be analysed individually to assess the potential harm that they could cause. It is worth noting that these compounds typically have relatively short half-lives in the environment and if they are not continuously released, it is quite likely that they will not pose any harm. This is because they will be rendered harmless and will be eliminated from the environment long before they will be able to cause any noticeable, detectable or significant damage. The toxicity of the breakdown products should be considered, however.

The ECP classes of contrast media and diuretics pose a relatively insignificant hazard to both human health and the environment based on the ranking of the chemicals that constitute these classes. The same can be said for ARVs and antivirals as these classes also feature chemicals that had rankings indicative of little or no harm. It is also worth noting that compared to the other ECP classes, relatively few international studies reported on these chemicals and the studies that did, prioritised these chemicals individually and did not consider other ECP classes. None of the chemicals found in the aforementioned classes had a rank lower than 78. This means that these chemicals are well outside the upper 50% of the analysed ECPs in terms of their final hazard-based rankings.

Phthalates also did not have any chemicals with a final ranking of less than 50, as their lowest ranking is the chemical benzyl butyl phthalate, which had a total final ranking of 53. It is worth noting that three of the remaining six phthalates analysed had total rankings between 60 and 70, which means that they can be considered borderline cases as they are located right on the edge of the upper 50% of the analysed ECPs. Further analysis of the potential hazard posed by phthalates to both human and environmental health should be done if possible, especially seeing as they are known carcinogens, although carcinogenicity was not part of the analysis process in this study. Carcinogenicity was not considered, as this study aimed to focus solely on the toxicity of the chemicals it analysed. It should also be mentioned that inclusion of carcinogenicity would have conflicted with the ranked list this study aimed to provide, as there are no varying levels of carcinogenicity that would allow for appropriate differentiation between substances.

The chemicals that constitute the “additional substances” class of ECPs comprised a wide array of chemicals with different functions and purposes. The chemicals cannot be divided into the other classes of ECPs and as such they were grouped together. They were indicated to be priority substances based on various literature reports and that is the primary reason behind their inclusion. These chemicals need to be analysed individually to determine the potential for harm that each possesses. Nine of the chemicals included in this class ranked within the top 50% of chemicals that were analysed. These chemicals were as follows: cinchonidine (30), cinchonine (24), clotrimazole (22), diphenylamine (49), diuron (55), ifosfamide (40), nicotine (29), tamoxifen (49) and telmisartan (37). These chemicals will thus be treated as high priority ECPs based on the rankings they obtained. Their continued study and monitoring is highly recommended.

Limitations to the hazard-based analysis included the limitations of the PBT profiler programme that was used to predict the aquatic half-life of the ECPs. This programme is unable to predict these half-lives with an optimal amount of accuracy as it assigns pre-set values to the chemicals. The chemicals cannot obtain unique values as they would likely have. The programme assigns values that are akin to intervals. These intervals were at 8.7, 15, 30, 60, 75 and 180 days respectively. All ECPs were assigned the values most representative of what the actual degradation time would likely be based on its chemical structure. As these values were used to obtain ranks for the ECPs, it created a scenario where numerous ECPs all achieved the same rank despite the possibility that they might not have an identical aquatic half-life when they occur in the environment. This can lead to the data obtained from the PBT profiler being unrepresentative of the actual *in situ* data. This can lead to an overall skewing of the data and it can make the process of accurate analysis and interpretation difficult. It should be noted, however, that these limitations are very unlikely to have a large impact on the overall ranking of the compounds. This is because compounds that have a long half-life will still fall in the interval given the highest priority and chemicals that were divided into the 15-day interval, for example, will still remain in the environment for a much shorter amount of time than chemicals that fall within the 60-day interval. This holds true even, for example, if the actual chemicals were to have real half-lives of 13 days and 65 days respectively.

The accuracy of the other data (partition coefficients and toxicity) can also be a concern since different sources can report different results. As the values directly influence the final ranking obtained by a specific chemical, data inaccuracies can negatively influence the results of the hazard-based analysis. Using acute toxicity data is also not optimal as most of these chemicals tend to occur in the environment at concentrations far below the levels required for acute toxicity to become a real concern. Many are also continuously being released into the environment and as such they gain pseudo-persistent effects. This means that chronic toxicity values and studies would be far more useful to assess the actual hazard posed by ECPs. However, there is a severe lack of chronic toxicity data available, which makes its usage near impossible.

It should also be mentioned that the toxicity data that is available has not been universally conducted on the same test species, which makes a comparison of these results challenging. To avoid this problem, only oral toxicities to rats were prioritised in this study. Some of the ECPs (predominantly those of the PAHs) lacked the required toxicity data that was used to compare the other chemicals. Toxicity equivalency scores can be used to compare these chemicals among themselves, but some of them could not be analysed during the main hazard-based prioritisation approach due to their aforementioned lack of data. Many PAHs also lacked data in terms of their half-lives in water, as the PBT profiler was unable to analyse them and could thus not predict their half-lives. This made their analysis via the hazard-based prioritisation approach completely impossible. It must be mentioned that as the hazard was calculated based on toxicity alone, other pertinent factors that can greatly increase the potential risk and hazard posed by a substance such as carcinogenicity and potential for endocrine disruption were not analysed. The omission of endocrine disruption as a factor was due mainly to a lack of data as the endocrine-disrupting capability of all the chemicals analysed in this study was not known at the time when the study was conducted.

3.7.2 ECPs in South African Water Bodies

Table 4 provides an overview of studies that have found ECPs in South African water bodies. From the table it becomes apparent that although research on ECPs in South African water systems is lacking, some research on these chemicals has been conducted and they have been found to be present in numerous water sources, including drinking water in certain cases. From Table 4 it also becomes apparent that most of the research has been conducted relatively recently as historical water pollution monitoring in South Africa was centred on more conventional and well-known sources and forms of water pollution.

Table 4 also shows that pesticides are the group of compounds whose presence in water resources has been most frequently reported in a South African context. This conclusion was based on the extensive list of pesticides that formed part of the prioritisation process that were also found in South African water resources by other studies. The large number of pesticides whose presence has been determined in South African water bodies is likely a direct result of the large size of the agricultural sector in South Africa. The size of this sector leads to the frequent widespread use of pesticides in order to assume high crop production. This increases the chances of these pesticides ending up in water resources thus it follows logically that such an extensive list of pesticides has been found to be present in South African water bodies. The high toxicity scores that are typical of this compound class also leads to numerous studies being conducted on them.

Antibiotics were the second most frequent class of compounds found in South African water bodies. It can safely be assumed that the use and thus presence of antibiotics in South African water bodies will vary seasonally. Thus, studies to locate antibiotics will likely be more successful in the colder winter months than in the warmer summer months as the general population uses more antibiotics during the winter months due to colds and flu. The increased occurrence of antibiotics in the environment during the colder winter months has been reported in numerous studies, which include a review study conducted by Kümmerer (2009). Since this project was a second-tier prioritisation approach, it can be assumed that many compounds including antibiotics would have already been excluded within the initial prioritisation approaches. Therefore, all the substances found in South African water bodies could not be included in Table 4. This could potentially skew some of the deductions made from it regarding the substances or ECPs in general; however, the statements that were made are valuable when it comes to the substances that were prioritised in this study in general. As such, it is of value to note that although research surrounding ECPs are lacking, the studies looking for ECPs in South African water bodies seemed to focus their attention on the correct substances as studies on pesticides in South African water bodies are numerous when compared with other compound classes.

Table 4: ECPs in South African Water Systems

Substance	South African reference(s)	Sampling medium	Rank obtained in this study
Analgesics and anti-inflammatory drugs			
Acetaminophen	(Odendaal et al., 2015) (Agunbiade & Moodley, 2014) (Matongo et al., 2015)	Drinking water, fresh water systems	88
Benzocaine	(Odendaal et al., 2015)	Drinking water	82
Diclofenac	(Agunbiade & Moodley, 2014)	Fresh water systems	11

Substance	South African reference(s)	Sampling medium	Rank obtained in this study
Ibuprofen	(Amdany, Chimuka & Cukrowska, 2014) (Agunbiade & Moodley, 2014) (Matongo et al., 2015)	WWTPs, fresh water systems	43
Ketoprofen	(Agunbiade & Moodley, 2014)	Fresh water systems	34
Naproxen	(Amdany, Chimuka & Cukrowska, 2014)	WWTPs	23
Antibiotics			
Ciprofloxacin	(Agunbiade & Moodley, 2014)	Fresh water systems	96
Erythromycin	(Agunbiade & Moodley, 2014) (Matongo et al., 2015)	Fresh water systems	79
Fluconazole	(Odendaal et al., 2015)	Drinking water	70
Metronidazole	(Matongo et al., 2015)	Fresh water systems	95
Nalidixic acid	(Odendaal et al., 2015) (Agunbiade & Moodley, 2014)	Drinking water, fresh water systems	72
Sulfamethazine	(Matongo et al., 2015)	Fresh water systems	109
Sulfisomidine	(Odendaal et al., 2015)	Drinking water	87
Sulfamethoxazole	(Agunbiade & Moodley, 2014) (Matongo et al., 2015)	Fresh water systems	98
Tetracycline	(Agunbiade & Moodley, 2014)	Fresh water systems	80
Trimethoprim	(Matongo et al., 2015)	Fresh water systems	95
Beta blockers			
Atenolol	(Agunbiade & Moodley, 2014)	Fresh water systems	91
Neuroactive compounds			
Carbamazepine	(Odendaal et al., 2015) (Burger & Nel, 2008) (Matongo et al., 2015)	Drinking water, freshwater systems	79
Temazepam	(Odendaal et al., 2015)	Drinking water	74
Psycho-stimulants			
Caffeine	(Naude et al., 2015) (Agunbiade & Moodley, 2014) (Matongo et al., 2015)	Freshwater systems	62
Disinfectants			
Chloroform	(Amdany, Chimuka & Cukrowska, 2014)	WWTPs	60
Triclosan	(Amdany, Chimuka & Cukrowska, 2014)	WWTPs	61

Substance	South African reference(s)	Sampling medium	Rank obtained in this study
Phthalates			
Bis[2-ethylhexyl] phthalate	(Fatoki et al., 2010)	Freshwater systems (Venda)	65
Dibutyl phthalate	(Fatoki et al., 2010)	Freshwater systems (Venda)	69
Diethyl phthalate	(Fatoki et al., 2010) (Naude et al., 2015)	Freshwater systems	89
Dimethyl phthalate	(Fatoki et al., 2010) (Naude et al., 2015)	Freshwater systems	93
Pesticides			
2,4-Dichlorophenoxyacetic acid [2,4-D]	(Burger & Nel, 2008)	Freshwater systems	33
Acetochlor	(Burger & Nel, 2008)	Freshwater systems	44
Aldicarb	(Burger & Nel, 2008)	Freshwater systems	25
Aldrin	(Burger & Nel, 2008)	Freshwater systems	2
Atrazine	(Odendaal et al., 2015) (Burger & Nel, 2008)	Drinking water, freshwater systems	21
Chlorpyrifos	(Burger & Nel, 2008) (Bennet et al., 2003) (Dabrowski et al., 2003) (London et al., 1995a) (Naude et al., 2015)	Freshwater systems	6
Cypermethrin	(Burger & Nel, 2008)	Freshwater systems	5
Dichlorodiphenyldichloro-ethane	(Heath & Claassen, 1999) (Sereda & Meinardt, 2003)	Freshwater systems	5
DDE	(Heath & Claassen, 1999) (Sereda & Meinardt, 2003)	Freshwater systems	19
DDT	(Heath & Claassen, 1999) (Sereda & Meinardt, 2003)	Freshwater systems	4
Deltamethrin	(Burger & Nel, 2008) (Sereda & Meinardt, 2003) (Dalvie et al., 2003) (Dabrowski et al., 2003)	Freshwater systems	1
Dieldrin	(Bouwman et al., 2003) (Heath & Claassen, 1999)	Freshwater systems	11
Endosulphan	(Burger & Nel, 2008) (Bennet et al., 2003) (Dalvie et al., 2003) (London et al., 2000)	Freshwater systems	10
Enilconazole	(Odendaal et al., 2015)	Drinking water	18
Heptachlor	(Heath & Claassen, 1999)	Freshwater systems	8

Substance	South African reference(s)	Sampling medium	Rank obtained in this study
Hexazinone	(Odendaal et al., 2015)	Drinking water	77
Imidacloprid	(Odendaal et al., 2015)	Drinking water	56
Lindane	(Burger & Nel, 2008) (Heath & Claassen, 1999) (Fatoki et al., 2010)	Freshwater systems	14
Metazachlor	(Odendaal et al., 2015)	Drinking water	57
Metolachlor	(Odendaal et al., 2015)	Drinking water	68
Simazine	(Odendaal et al., 2015) (Burger & Nel, 2008)	Drinking water, freshwater systems	58
Tebuthiuron	(Odendaal et al., 2015)	Drinking water	53
Vinclozolin	(Burger & Nel, 2008)	Freshwater systems	80
PAHs			
Acenaphthene	(Naude et al., 2015)	Freshwater systems	54
Acenaphthylene	(Naude et al., 2015)	Freshwater systems	58
Fluoranthene	(Naude et al., 2015)	Freshwater systems	49
Fluorene	(Naude et al., 2015)	Freshwater systems	57
Naphthalene	(Naude et al., 2015)	Freshwater systems	29
Phenanthrene	(Naude et al., 2015)	Freshwater systems	50
Pyrene	(Naude et al., 2015)	Freshwater systems	55
Additional substances			
Cinchonidine	(Odendaal et al., 2015)	Drinking water	30
Cinchonine	(Odendaal et al., 2015)	Drinking water	24
Diphenylamine	(Odendaal et al., 2015)	Drinking water	49
Ephedrine	(Odendaal et al., 2015)	Drinking water	60
Minoxidil	(Odendaal et al., 2015)	Drinking water	73
Phenytoin	(Odendaal et al., 2015)	Drinking water	66
Telmisartan	(Odendaal et al., 2015)	Drinking water	37
BFRs			
Decabromodiphenyl ether	(Naude et al., 2015)	Freshwater systems	32
Hexabromocyclododecane	(Naude et al., 2015)	Freshwater systems	57
Octabromodiphenyl ether	(Naude et al., 2015)	Freshwater systems	48
Pentabromodiphenyl ether	(Naude et al., 2015)	Freshwater systems	51

A study completed in 2012 (Olukunle et al., 2012) confirmed the presence of BFRs in South African water bodies by finding these ECPs in the sediment of the Jukskei River in Gauteng. The highest concentration of BFRs within the Jukskei River was found at Eastgate in Alexandra (Olukunle et al., 2012). The elevated reading in this region was attributed to the high amount of run-off within this area in conjunction with poorly managed refuse dumps associated with the informal settlements along the riverbanks (Olukunle et al., 2012). It is worth noting that all the BFR concentrations in the water samples were below the detection limits of the equipment used in this study (Olukunle et al., 2012).

Table 5 contains information on most of the prioritised PAHs toxic equivalency scores. This was necessitated so that PAHs could be compared with each other in terms of toxicity. The PAHs that lack a toxic equivalency score all features more than two fused rings and have a low partition coefficient, which means that they are unlikely to remain in the water for extended periods of time. This means that even if they are toxic, it is unlikely that they will be found in the water and are thus more likely to accumulate in the sediment.

From Table 5 it is apparent that benzo[a]pyrene is the most toxic PAH by a significant margin. However, it should be noted that it is the carcinogenicity of PAHs that is more cause for concern than their toxicity. However, as the prioritisation approach within this study relied upon toxicity, it is of value to analyse the toxic equivalency of PAHs as they lack toxicity information. This enables the comparison of PAHs; however, they still cannot be compared to other substance in terms of toxicity. This does place limitations on the amount of comparison, ranking and prioritisation that are possible for the PAH class of compounds within the larger group of ECPs due to current toxicity data limitations.

Table 5: Toxic equivalency of prioritised PAHs

PAH	Toxic Equivalency	Reference	Number of Fused Rings
Benzo[a]pyrene	1	(Nisbet & Lagoy, 1992)	5
Acenaphthene	0.001	(Nisbet & Lagoy, 1992)	2
Acenaphthylene	0.001	(Nisbet & Lagoy, 1992)	2
Anthracene	0.01	(Nisbet & Lagoy, 1992)	3
Benz[a]anthracene	0.1	(Nisbet & Lagoy, 1992)	4
Benzo[b]fluoranthene	0.1	(Nisbet & Lagoy, 1992)	4
Benzo[c]fluorene			3
Benzo[ghi]perylene	0.01	(Nisbet & Lagoy, 1992)	6
Benzo[j]fluoranthene			4
Benzo[k]fluoranthene	0.1	(Nisbet & Lagoy, 1992)	4
Chrysene	0.01	(Nisbet & Lagoy, 1992)	4
Cyclopenta[cd]pyrene			4
Dibenz[a,h]anthracene	0.1	(Nisbet & Lagoy, 1992)	5
Dibenzo[a,e]pyrene			5
Dibenzo[a,h]pyrene			4
Dibenzo[a,i]pyrene			6
Dibenzo[a,l]pyrene			5
Fluoranthene	0.001	(Nisbet & Lagoy, 1992)	3
Fluorene	0.001	(Nisbet & Lagoy, 1992)	2
Indeno[1,2,3-cd]pyrene	0.1	(Nisbet & Lagoy, 1992)	5
Naphthalene	0.001	(Nisbet & Lagoy, 1992)	2
Phenanthrene	0.001	(Nisbet & Lagoy, 1992)	3
Pyrene	0.001	(Nisbet & Lagoy, 1992)	4

3.7.3 Discussion of the GIS results (potential sampling site selection)

The GIS results obtained from this study provides a clear indication of where the biggest potential problem areas regarding ECPs (both anthropogenic and agricultural) lie within the province of Gauteng. ECPs are likely to be found within the indicated river sections. Therefore, the GIS results obtained by this study can serve as a valuable starting point for any sampling driven research that might be conducted in the future. The municipal wards that fall within the indicated high risk areas are listed in Appendix 1. These wards can use the results of this study in an attempt to mitigate or avoid potential harm to both ecosystems and the public exposed to these ECPs. Educating the public and garnering their support in attempts to minimise the amount of ECPs that end up in water resources could have positive effects in the future.

It is worth noting that the identified areas are by no means the only areas that are potentially exposed to ECPs. The identified areas are simply a representation of the areas most likely to be exposed to the highest amount of potential harm based on the parameters deemed important in this study (see GIS methodology). It is expected that other areas within the province are also at risk and that a thorough ECP sampling study will have to be conducted throughout Gauteng to determine more accurately which areas are most susceptible to harm and where mitigation efforts should be focused.

3.8 Conclusion

In general, ECPs pose a novel and potentially significant challenge to not only water resources, but also to other environmental compartments such as air, soil, ecosystems and even human health (Gavrilescu et al., 2014). The development and mass production of novel chemicals have been reported to often exceed the ambit of existing safety monitoring and risk assessment methods. The unexpected negative effects of these chemicals can also show the inadequacy of current preventative and remediation technologies and can have consequences such as irreversible environmental damage (Gavrilescu et al., 2014).

Continued advances in technology coupled with increases in the global population are likely to cause a decrease in water availability, increased water reuse and, importantly, an increase in the concentrations of ECPs found in water bodies (Cizmas et al., 2015). This leads to the conclusion that further research regarding ECPs and the potential risk they pose to humans and the environment is imperative. Research into improving the technology of WWTPs and increasing their efficiency at removing all classes of ECPs from waste water is also required (Cizmas et al., 2015). Lastly, standardising risk assessment methodologies and monitoring methodologies will also make data more universally comparable and will greatly aid research and help to inform decision makers (Cizmas et al., 2015).

When assessing the threat that a specific ECP can pose after a sample has been obtained, there are certain considerations for an accurate assessment to be completed. These considerations are (Gavrilescu et al., 2014):

- Contaminant concentration.
- Contaminant characteristics and category or compound class under which the chemical falls (for example, whether the chemical is an antibiotic or a pesticide).
- Scale and level of contamination.
- The risk intensity generated for health or the environment.
- The opportunity for the threat assessment to be applied *in situ* or *ex situ*.
- The later use of the site.
- Available resources.

A holistic, multi-disciplinary approach is recommended to evaluate the potential threats posed by ECPs and also with respect to the removal of these pollutants from a specific environment (Gavrilescu et al., 2014).

Garica et al. (2011) reported that both acute and chronic ecotoxicity tests surrounding ECPs need improvement. The study stated that more *in situ* data measurement of ECPs is also required in an attempt to create more accurate predicted environmental concentrations. This study found that 53.8% of the PCPs examined (14 of a total of 26 chemicals) were harmful to aquatic life based on ecotoxicity testing. This confirms that certain ECPs possess the potential to cause harm in the environment. Thus, this study serves to illustrate the need for further testing and refinement of methodologies to determine the risk and hazard posed by ECPs in the environment (García et al., 2011).

Species-specific toxicity testing for ECPs are required as different species might react differently to the various chemicals (Cizmas et al., 2015). A certain species might be more sensitive to one chemical while another species might be more sensitive to a different chemical. This clearly illustrates the need for increased species-specific research (Cizmas et al., 2015). Additionally, the effect that the mixing of compounds has on the toxicity must also be examined as these chemicals never occur alone in the environment; the combination of chemicals could potentially have worse effects than the effect of a single chemical (Cizmas et al., 2015). If this is the case, the risk these chemicals pose could potentially be underestimated. A study completed in 2015 (Herrmann et al., 2015) recommended that further research is required in terms of the risk posed by neurological drugs on the environment as there is not any conclusive evidence that neurological drugs are harmless once they enter natural water bodies.

From the results and discussion sections of this report, it becomes apparent that of the ECPs considered, pesticides pose the greatest potential hazard based on the approach used in this study. Pesticides are of particular concern in South Africa due to the frequent and widespread usage thereof owing to the scale of the agricultural sector within the country. Pesticides as a class of ECPs pose substantial potential harm to ecosystems and the environment throughout the country. Provinces that have a higher use of pesticides, such as the Free State (Dabrowski et al., 2014), will potentially be exposed to greater harm. Therefore, it is imperative that future research regarding the potential detriment that pesticide usage can entail for both humans and the environment should be conducted to improve the understanding of the potential hazards and gain insight into possible mitigation measures and solutions.

The creation of a universally accepted standard definition surrounding what exactly an ECP is and what compounds form part of this definition will greatly aid in the science of analysing and studying ECPs. Currently, there is disparity regarding what exactly constitutes an ECP and initial analyte lists of studies concerning ECPs are largely determined by the specifics of the project or by the researchers. This creates problems in terms of the comparison and universal validity of these studies. This would mean that future studies would not need to rely on calculated data, but could use actual measured data, which would reduce the limitations of the studies. Including full data on MSDSs would also prove useful to all the industries that work frequently with these chemicals.

The literature review regarding occurrence information surrounding ECPs in South African water bodies has found that there is a lack of data available in this country. As such, the creation of a database that contains pertinent information surrounding the occurrence, toxicity (especially chronic toxicity), persistence and bioaccumulative potential of all ECPs would provide an invaluable resource from both a scientific and environmental point of view. This database should be in the public domain and scientists from across the country should be granted easy access it.

The hazard-based prioritisation method used in this study is not without its shortcomings and limitations; however, it provides useful insight into which ECPs may pose a greater threat than others. From this study, the scientific reasoning behind which ECPs warrant further investigation as a consequence of their final priority ranking can be acquired. This study can prove to be a valuable stepping stone for other studies wishing to investigate ECPs and the potential harm that these substances could entail for human health as well as for the environment.

4 FLUORESCENCE- AND NANOMATERIAL-BASED SENSORS FOR MONITORING SELECTED ORGANIC POLLUTANTS IN WATER

By Hanieh Montaseri, Sifiso Nsibande and Patricia Forbes[8]

4.1 Introduction

Effective environmental monitoring programmes are an integral component of environmental management of natural resources, including water. ECPs comprise a wide spectrum of chemicals for which no legislative limits are currently in place, but which may affect the environment and human health (Liu et al., 2014b). Hutchinson et al. (2013) define ECPs as chemicals that have been detected in the environment, but which are currently not included in regulatory monitoring programmes and whose fate and biological impacts are poorly understood.

ECP compound classes include pesticides, PAHs, pharmaceutical and personal care products (PPCPs), flame retardants, perfluorinated compounds and industrial additives (Liu et al., 2014b), as shown in Table 6. ECPs are of particular environmental concern as regulatory and/or standard analytical methods have yet to be developed and they may affect water quality, ecosystems and human health. Therefore, they have become a new area of concern in environmental science. In addition, there is a paucity of accurate and precise data concerning their concentration, biodegradation resistance and their risks in the environment. A detailed background to ECPs and compounds within each compound class is provided in Chapter 2 of this report and is therefore not expanded on here in detail.

Generally, environmental monitoring is a process that involves the identification of selected variables of the environment and subsequently quantifying them (Gupta, 2011). Simple analytical probes are often used in environmental monitoring since they are cheaper, cater for on-site real-time monitoring, and are easier to handle than laboratory measurements using conventional instruments. Different working mechanisms are adopted in these probes depending on the detection principle. Optical measurement methods have gained attention due to advantages including small size, high sensitivity, ease of implementation, and generally fast response times (Hung, 1982; Lee, 2003). Fluorescence measurement techniques are a popular subset of optical methods, which use a fluorophore material as a sensing receptor to detect a specific analyte. The type of fluorophore can be chosen from a vast selection of nanomaterials including carbon nanoparticles, gold nanoparticles (AuNPs), magnetic nanoparticles and semiconductor QDs (Liu et al., 2014b).

The primary aim of this research is to develop photonic sensor materials to detect ECPs in water systems. In order to demonstrate the potential of such a monitoring approach, this project serves to demonstrate the proof of concept via the development for selected ECPs within the compound classes of pharmaceuticals, PPCPs, pesticides and PAHs. This report serves to review the literature applicable to these selected target analytes with respect to the development of fluorescence sensor materials and related sensor methods based on nanomaterials, with particular reference to QDs. An introduction to these materials and an overview of the published means of synthesising them are also presented. In some cases where fluorescence- and/or nanomaterial-based sensor methodology is scarce, alternative methods are briefly mentioned for comparison purposes.

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Table 6: Examples of emerging compound classes (Lopez de Alda et al., 2003)

Compound class	Examples
Veterinary and human antibiotics	Trimethoprim, erythromycin, lincomycin, sulfamethoxazole
Analgesics and anti-inflammatory drugs	Codeine, ibuprofen, acetaminophen, acetylsalicylic acid, diclofenac, fenoprofen
Psychiatric drugs	Diazepam
Lipid regulators	Bezafibrate, clofibric acid, fenofibric acid
β-Blockers	Metoprolol, propranolol, timolol
X-ray contrasts	Iopromide, iopamidol, diatrizoate
Steroids and hormones (contraceptives)	Oestradiol, estrone, estriol, diethylstilbestrol
PCPs:	
Fragrances	Nitro, polycyclic and macrocyclic musk
Sunscreen agents	Benzophenone, methylbenzylidene camphor
Insect repellents	*N*,*N*-Diethyltoluamide
Antiseptics	Triclosan, chlorophene
Surfactants and surfactant metabolites	Alkylphenol ethoxylates, alkylphenols (nonylphenol and octylphenol), alkylphenol carboxylates
Flame retardants	Polybrominated diphenyl ethers (PBDEs), tris(2-chloroethyl)phosphate
Industrial additives and agents	Chelating agents (EDTA), aromatic sulfonates
Gasoline additives	Dialkyl ethers, Methyl-t-butyl ether (MTBE)
Disinfection by-products	Iodo-trihalomethane, bromo acid, bromoacetonitrile, bromoaldehyde, cyanoformaldehyde, bromate
PAHs	Pyrene, phenanthrene, benzo[a]pyrene
Pesticides	Atrazine, terbuthylazine, glyphosate

4.2 Quantum Dots

QDs are semiconductor crystalline nanomaterials that have unique electronic and optical properties due to quantum confinement effects (Murphy & Coffer, 2002). They have many attractive properties that make them suitable as analytical sensors. Through surface modification strategies they have found various applications across disciplines. Reviews have been published on their application in solar cells (Nozik et al., 2010), as biological labels (Jin et al., 2011), in microelectronics (De Menezes et al., 2005) and in electrochemistry (Huang & Zhu, 2013). This report presents an overview of different studies where QDs have been applied as sensors for organic pollutants, with emphasis on fluorescence detection of PAH compounds, pesticides, triclosan (as an example of a PCP) and acetaminophen (a commonly used pharmaceutical).

A QD is a semiconductor nanocrystal with physical dimensions smaller than the exciton Bohr radius (Noh et al., 2010). The cores are typically made from elements from group II and VI [indium phosphate (InP), indium arsenate (InAs), gallium arsenate (GaAs) and gallium nitride (GaN) metalloid cores] or groups III and V such as zinc sulphide (ZnS), zinc-selenium (ZnSe), cadmium-selenium (CdSe), and cadmium tellurium (CdTe) (Dabbousi et al., 1997). The QD cores are small enough to exhibit quantum mechanical properties due to confinement of the excitons in all three spatial dimensions. The electronic properties of these materials are intermediate between those of bulk semiconductors and of discrete molecules (Brus, 2007; Murray et al., 2000; Norris, 1995). Alexey Ekimov first discovered QDs in 1981

(Ekimov et al., 1985; Ekimov & Onushchenko, 1982) in a glass matrix followed by Louis E. Brus in 1985 in colloidal solutions (Allwine et al., 2002). The term “quantum dot” was coined by Mark Reed (1988).

QDs have unique chemical and physical properties including size-dependent fluorescence, high fluorescence quantum yields, narrow spectral line widths, independence of emission on the excitation wavelength, and stability against photobleaching (William et al., 2006). Furthermore, because of their large surface-to-volume ratios, their surface activity and their strong adsorption affinity to other nanoparticles, their use can be advantageous over larger particles. QDs have size-dependent optical properties, which mean their absorption and emission properties can be tuned by changing the particle size, shape and surface structure (Zhang et al., 2003). Figure 12 illustrates how the emission wavelengths of QDs vary with particle size.

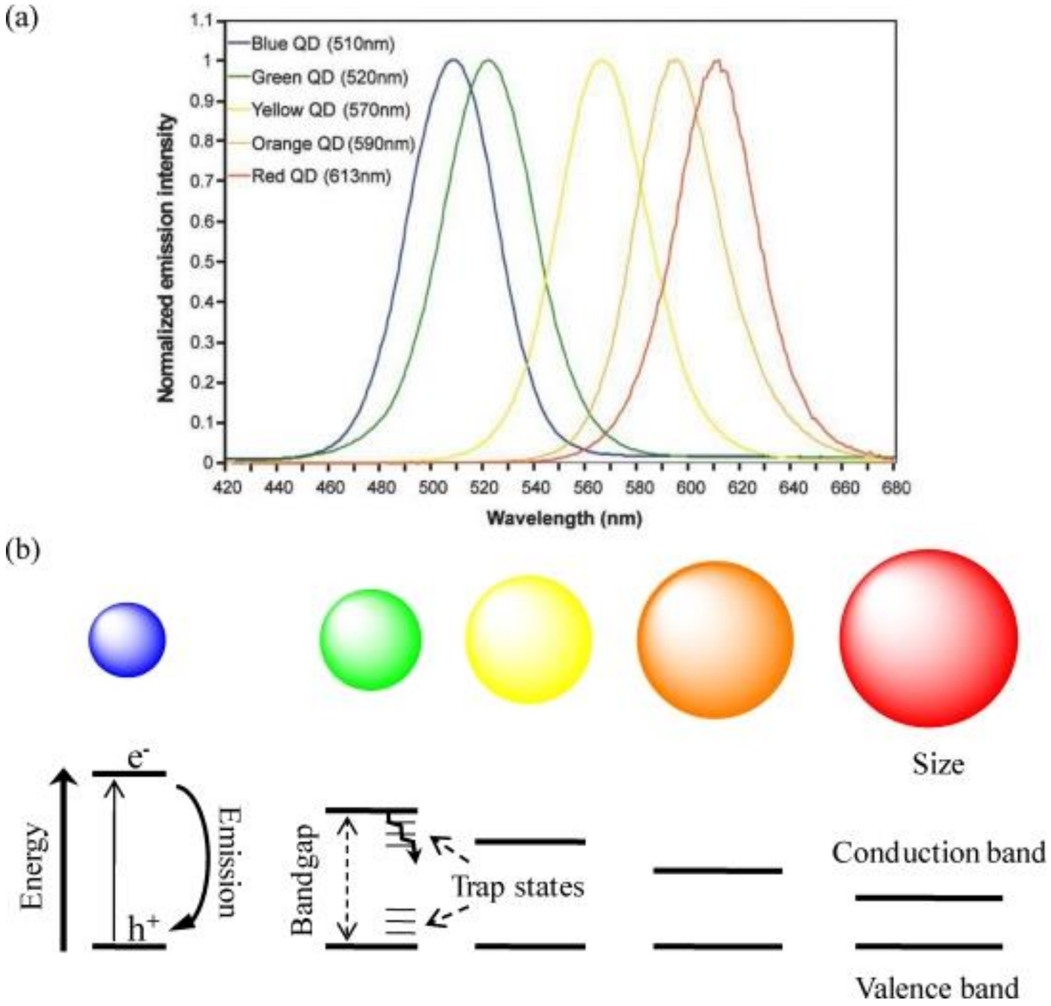


Figure 12: (a) Tunable emission wavelength arises from changing the particle size; (b) Creation of exciton upon photon absorption followed by fluorescence emission or relaxation through trap states (Frasco & Chaniotakis, 2009; Jaiswal et al., 2003)

QDs have unique physicochemical properties that pertain to the combination of their crystalline metalloid core structure/composition and quantum-size confinement (Hardman, 2006). In addition, when the size of the exciton, given by the exciton Bohr radius [rBohr (~1–5 nm)], exceeds the physical size of the semiconductor nanocrystal (*D*), the quantum confinement effect of QDs occurs, which provides size-dependent emission (Wilson et al., 1993). Semiconductor nanocrystals exhibit particularly interesting properties when the exciton is strongly confined (D < 2 rBohr) (Martienssen & Warlimont, 2005). Also, QDs are photo-electrochemically active and they have potential to be applied in (photo) electrochemical sensors (Gill et al., 2008). These properties enable the use of QDs as reliable optical labels in environmental analysis.

4.2.1 Synthesis and characterisation of QDs

Crystalline QD nanoparticles are usually prepared from group II–VI or group III–V elements in the periodic table using different approaches that have been comprehensively discussed in previous reviews (Bera et al., 2010; Drbohlavova et al., 2009; Murphy & Coffer, 2002). Synthesis techniques can be grouped into two main categories:

- Colloidal chemistry or the “bottom-up” approach, which involves injecting precursors into hot solutions under vigorous stirring and allowing nucleation to occur thereby producing the QDs as colloids.
- Epitaxial growth or nanoscale patterning “top-down” approach where QDs are produced lithographically or chemically from semiconductor substrates.

Colloidal chemistry is the preferred route because it is facile and can be performed in “one pot”. When this is carried out in low temperature polar solvents such as water and methanol, it is referred to as “wet chemistry”. If non-polar organic solvents such as trioctylphosphine oxide (TOPO) are used, it is referred to as “organometallic synthesis”, which provides superior crystallinity and high PL quantum yield from higher temperature reactions. In either case, the size of the QDs synthesised is controlled by the reaction time. It is important to control the size, shape, homogeneity and surface structure when synthesising QDs to achieve the desired properties of the resultant nanoparticles. In the first step of the QD synthesis, precipitation from a solution containing metal ions such as Cd, Ag, Zn and In by hydroxides of S, Se, or Te is performed (Farkhani & Valizadeh, 2014).

There are several characterisation techniques used to confirm the synthesis of QD materials (Drbohlavova et al., 2009). The morphology and size distribution of QD nanoparticles can be studied using microscopy techniques like transmission electron microscopy (TEM) and by investigating powder X-ray diffraction (XRD) patterns. Information on the size, optical, and functional properties of the material can be studied using techniques such as absorption, fluorescence spectroscopy and fluorescence lifetime measurements. For a particular QD, different optical properties can be obtained by controlling their size during synthesis.

4.2.1.1 Organometallic synthesis of QDs

Organometallic synthesis of QDs was invented by Steigerwald and Brus (1990) and it is a widely used approach. It involves preparation of QDs in high temperature organic solvents. The reaction mixture comprise three components: precursors, organic surfactants and solvents; although occasionally, surfactants can act as solvents (Yin & Alivisatos, 2005).

Different sized QDs can be manufactured by changing the number of precursors and growth time. An important part of synthesis is finding the appropriate precursor molecules to serve as organometallic reagents as they are needed to decompose and produce reactive atomic or molecular species (monomers) that influence nanocrystal nucleation and growth. In the synthesis of chalcogenide QDs, cadmium precursors are organometallics (Hambrock et al., 2001) like CdO or $Cd(CH_3)_2$ whereas Se, S and Te precursors are usually from elemental sources. Organic solvents include TOPO (Murray et al., 1993), hexadecyl amine (Talapin et al., 2004) and octadecene (Bullen & Mulvaney, 2004), which may be used at high temperature (200–400°C). They are chemically bonded to the precursors by Van der Waals interactions. In this process, organometallic precursors in hot coordinating solvents are pyrolysed to produce monodispersed QDs with $< 5\%$ size distribution (Murray et al., 2000). Reactive species known as monomers are manufactured by dissolving and decomposing precursors in the solvent. The best precursors contain “leaving groups” that quickly depart to leave the favourable reactive species (Yin & Alivisatos, 2005).

Two phenomena should occur in this procedure:

- Decomposition or reaction of precursors at high temperature to generate a supersaturated solution of monomer followed by a burst of nucleation of nanocrystals.
- Growth of nuclei from molecular precursors.

The reaction typically is performed under an inert gas such as Ar since some nanocrystals are sensitive to air and also reactant molecules may be pyrophoric (Farkhani & Valizadeh, 2014). Organometallic precursors produce CdSe, CdS or CdTe QDs, but they have low quantum yield (< 10%) and the bare QDs can be easily oxidised, which results in the release of toxic ions. These QDs must therefore be passivated or covered with a shell of higher band-gap material such as ZnS, which is grown epitaxially around the core to increase the quantum yield up to 80%. It is noteworthy to mention that photostability and biocompatibility have been dramatically improved via epitaxial growth (Hines & Guyot-Sionnest, 1996; Li et al., 2003). High reaction temperature for the synthesis of QDs has considerable advantages: it can facilitate the removal of crystalline defects and allow PL improvement (Cingarapu et al., 2012).

Moreover, QDs synthesised by this method have high quantum yields, and size distribution can be controlled easily by changing the reaction time (Bakar et al., 2008) and temperature. More importantly, QDs with a narrower particle size distribution can be manufactured (Gaponik et al., 2002).

4.2.1.2 Water solubilization of QDs

The QDs synthesised by organometallic methods are soluble for use in non-polar organic solvents (Smith et al., 2008). Hence, they must be modified to be soluble in water and be compatible for environmental analysis. Two strategies have been developed for this purpose:

- Ligand exchange by replacing the hydrophobic ligands with new ligands containing thiol groups and aminated polymers.
- The surface can be modified by encapsulation within block polymer shells and phospholipid micelles.

In encapsulation, amphiphilic molecules (Smith et al., 2008) such as β-cyclodextrine (β-CD) and polymer microspheres (Cassette et al., 2013; Mattoussi et al., 2012) have been used. In addition, silica-coating makes QDs water soluble.

4.2.1.3 Wet chemistry

The application of direct aqueous synthesis of QDs such as CdSe (Rogach et al., 1999) and CdTe (Gaponik et al., 2002) using thiol groups as stabilisers can provide an alternative route instead of using high boiling organic solvents. In this procedure, exchange processes are needed and the ligands used during the synthesis become surface ligands (Zhang & Clapp, 2011). In aqueous routes, a metal salt and a thiol compound are first dissolved in water. Heating of the reaction mixture allows particle growth to commence, similar to organometallic synthesis (Eychmüller & Rogach, 2000). In spite of the fact that the wet chemistry approach is environmentally friendly, cheap and reproducible, and it is suitable for large-scale synthesis (Li et al., 2011), some serious drawbacks have restricted the use of these QDs. First, they are apt to degradation and aggregation in solution because of weakly bound thiol ligands. Secondly, they suffer from relatively poor photostability (Law et al., 2009). In addition, the salt tolerance of prepared QDs in the aqueous phase is lower than QDs prepared in an organic phase (Liu et al., 2010).

4.2.2 Toxicity considerations

Despite the important properties of QDs and their invaluable social benefits such as drug targeting and *in vivo* biomedical imaging (Gao et al., 2004; Michalet et al., 2005), their potential toxicity have restricted their application. QD toxicity depends on multiple factors resulting from individual QD physicochemical properties and environmental conditions including QD size, charge, concentration, outer coating bioactivity (capping material and functional groups), and oxidative, photolytic, and mechanical stability (Hardman, 2006). They may also pose a threat to the environment and human health due to their very small size and strong adsorption ability, they can penetrate through skin and result in significant adverse health effects (Liu et al., 2014b). Therefore, it is critically important to develop a stable sensor from which the chemical components do not leach. The main concern for their toxicity is the release of heavy metals (e.g. Cd^{2+}) from the core of QDs (Chen et al., 2012a), because the cores of QDs are mostly synthesised from heavy metals. However, stabilisation of the core QD with an inorganic shell such as ZnS and also the surface coating with a capping agent [e.g. mercaptoacetic acid (MPA), L-cysteine (L-cys) and GSH] can reduce ion leakage and subsequent toxicity (Tiwari et al., 2011; Zhang et al., 2006b) and additionally protect the core from air oxidation (Su et al., 2009).

Studies have been done to investigate toxicity of different QDs in different living systems like cell cultures and mice (Valizadeh et al., 2012). It has thus been shown that passivating the core with non-toxic shell layers greatly reduces leakage of cadmium, thereby reducing potential toxic effects. For example, a study by Kuzyniak et al. (2014) spiked living cells with increasing concentrations of CdSe and the release of cytoplasmic enzyme (LDH) was used as a measure of toxicity. Their study showed that capping the QDs with the ligand GSH, and encapsulating with a non-toxic ZnS shell to form GSH-CdSe/ZnS nanoparticles, greatly reduced the toxicity of QDs in the living cells.

4.2.3 Core/shell nanoparticles

The terminology "core/shell" (Zhou et al., 1994) was established when researchers found that a heterogeneous composite or sandwich colloidal semiconductor particles have better efficiency than their corresponding single particles (Youn et al., 1988). They can be widely defined as a core (inner material) and a shell (outer layer material) (Ghosh Chaudhuri & Paria, 2012), which consist of a wide range of different combinations in close interaction. The purpose of the coating on the core particle is manifold, such as surface modification, the ability to increase the functionality, stability, and dispersibility, controlled release of the core, and reduction in consumption of precious materials (Ghosh Chaudhuri & Paria, 2012). The spectroscopic characterisation of the core/shell particles show that with increasing shell thickness, the intensity of the UV absorbance increases and the reflectance shifts towards the higher wavelength region (Ye et al., 2008). Because of the shell material coating, the properties of the core particle, such as reactivity, decrease or thermal stability can be modified so that the overall particle stability and dispersibility of the core particle increases.

Inorganic/inorganic core/shell nanoparticles are the most prominent class of core/shell nanoparticles. These types of particles are broadly applied for the improvement of semiconductor efficiency, information storage, optoelectronics, catalysis, optical bioimaging, and biological labelling. The concept of a multilayer semiconductor particle was first developed by Eychmüller et al. (1993). The distinct advantages of multilayer semiconductor nanoparticles are higher quantum yield, higher PL efficiency, improved optical properties, increased half-life times and easy detection of emission spectra because they are shifted towards higher wavelength in the visible range. Moreover, due to improved electronic properties (band gap and band alignment), they have better structural (lattice mismatch) properties than core QDs.

In order to render synthesised core/shell QDs water soluble, they can be functionalised or given secondary coatings to improve water solubility, QD core durability, and suspension characteristics. Functionalisation may be achieved via electrostatic interactions, adsorption, multivalent chelation, or covalent bonding (Hardman, 2006).

4.2.4 Graphene QDs

Recently, graphene QDs (GQDs) have been applied as a class of zero-dimensional graphitic nanomaterials that are superior in terms of chemical inertness, ease of production, resistance to photo-bleaching, low cytotoxicity and excellent biocompatibility in comparison to conventional semiconductor QDs that make them favourable in sensors, bioimaging, optoelectronic devices and so on (Zhang et al., 2012; Zhu et al., 2012). Furthermore, similar to graphene, GQDs have excellent characteristics of a large surface area and fine surface grafting using the π-π conjugated network or surface groups and other special physical properties. It is noteworthy to mention that the carboxyl and hydroxyl groups at their edge enable them to display excellent water solubility and suitability for successive functionalisation with various organic, inorganic, polymeric or biological species (Luo et al., 2013b; Shen et al., 2011; Shen et al., 2012b; Tetsuka et al., 2012).

4.2.5 Molecular imprinted polymers

Molecular imprinted polymers (MIPs) are synthetic polymeric materials that have specific recognition sites complementary in shape, size and functional groups to the template molecule (Holthoff et al., 2011; Yang et al., 2011). In molecular imprinting, functional monomers are located around the template molecules by non-covalent interaction or reversible covalent interaction, followed by polymerization and the template removal. So a molecular ‘memory’ within the imprinted polymer matrix is created, and then the target molecule could be selectively distinguished based on the molecular 'memory' (Fang et al., 2009). MIPs have been considered as alternatives to natural receptors (Reddy et al., 2013; Yuan et al., 2011) in chemical assays or sensors due to their low cost, long lifetime, their tailor-made recognition sites for target analytes and intrinsic robustness. Figure 13 illustrates the synthetic route to generate fluorescent MIPs.

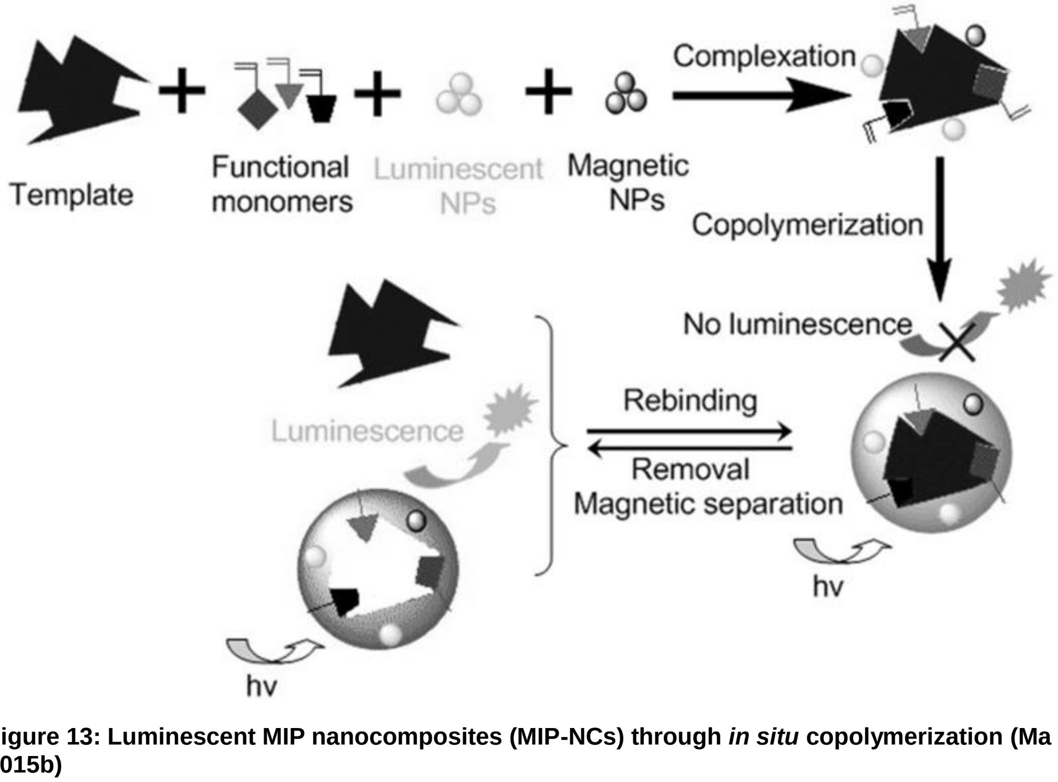


Figure 13: Luminescent MIP nanocomposites (MIP-NCs) through *in situ* copolymerization (Ma et al., 2015b)

4.2.6 Composite QD-based molecularly imprinted polymer

Due to their mechanical/chemical stability, easy preparation and low cost, MIPs have been widely used in a variety of applications, such as separation media (Zhang et al., 2006a), mimicking antibodies (Haupt & Mosbach, 2000; Wulff, 2002), selective removal, and determination of environmental pollutants (Meng et al., 2005; Pichon & Chapuis-Hugon, 2008). Quantum dot-based molecularly imprinted polymer (QD-MIP) merges the advantages of surface molecular imprinting and nanotechniques, where a surface molecular imprinting shell and nanocomposite core can enable the template-imprinting sites to be placed at the surface of the material to provide favourable selectivity, high capacity and fast association/ dissociation kinetics (Li et al., 2009b; Lu et al., 2007). One of the important factors for successful molecular imprinting is the presence of functional monomers in the polymer matrix.

A disadvantage of molecular imprinting technology is that analytes bound to the recognition sites of MIPs must be removed and then detected via expensive instrumentation such as gas chromatography–mass spectrometry (GC–MS), high-performance liquid chromatography (HPLC), or LC–MS (Li et al., 2006). In addition, after preparation of MIPs through *in situ* polymerization of functional monomers, excessive templates are needed to effectively increase recognition cavities in the MIP matrices, which conversely, would result in more detrimental environmental impact because more organic solvents are used to remove the templates from the MIPs. Overall, it is also difficult to prepare functional MIP receptors that can be applied to specifically recognise and quantify target analytes.

Although the preparation of MIPs based on hydrogen bonding has attracted attention, loss of recognition selectivity occurs in aqueous media. However, fluorescent signals may enhance the use of the MIP sensors without sacrificing accuracy or efficiency. Luminescent nanomaterials (Deng et al., 2011; Wang et al., 2010) enjoy good chemical stability, great photostability, and readily tunable spectral properties. Among various types of luminescent nanomaterials, QDs have drawn a great deal of research efforts for use *in vivo* or *in vitro* imaging, fluorescence immunoassays and toxin detection (Chan & Nie, 1998; Rhyner et al., 2006), as previously mentioned. Through a facile and versatile ultrasonication-assisted encapsulation method, QD-MIP composite nanospheres can be prepared.

Unlike the hydrogen-bond-based MIPs, QD-MIP composite nanospheres rely on interactions including Van der Waals forces and hydrophobic forces, and display excellent selectivity in aqueous media. In addition, their small particle sizes and carboxyl-enriched polymer matrices produce outstanding dispersibility and stability in aqueous solution. They also have faster adsorption and desorption kinetics, which allow them to be applied for chemical/biological sensors in aqueous media that reduce usage of organic solvents for washing (Zhao et al., 2012). In addition, almost all the templates are encapsulated into the QD-MIP nanocomposites, because of their relatively low dissolubility in water. Therefore, enough recognition sites can be produced from a relatively small amount of the template. Figure 14 presents a scheme for the formation of QD-MIP.

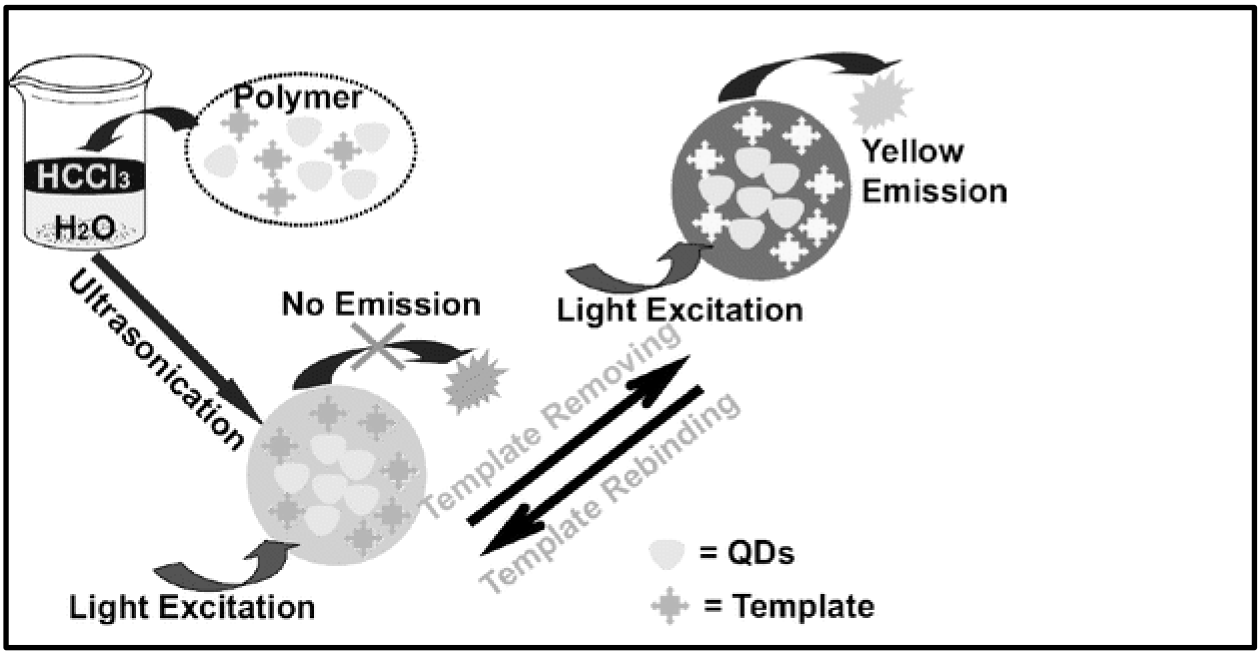


Figure 14: Scheme for the preparation of QDs-based molecularly imprinted polymer (QDs-MIP) nanospheres and the fluorescence quenching detection of analytes upon specific recognition. The copolymer, QDs, and templates were dissolved in chloroform in advance and then transferred into water. The QDs-MIP nanospheres were obtained via ultrasonication, evaporating the chloroform and removing the templates by washing with methanol in sequence (Zhao et al., 2012)

4.2.7 Principle of optical sensing using QDs

Sensing with QDs is based on the sensitivity of their luminescence to the surface state of the nanoparticles. Thus, sensing can result from interaction (chemical or physical) with the target analyte, which can lead to either PL enhancement or quenching (Murphy, 2002).

The basis of most QD sensing assemblies, however, is not direct interaction with the QD surface, but rather energy flow between the QDs and analyte molecules. Förster Resonance Energy Transfer (FRET) occurs when energy absorbed by a donor (usually the QDs) is transferred to a nearby acceptor species. The efficiency of this non-radiative energy transfer depends on (Chou & Dennis, 2015; Guo et al., 2014; Yang et al., 2010):

- The distance between the FRET pair (donor and acceptor).
- Special arrangement or orientations of the pair.
- The spectral overlap of the donor emission and acceptor absorption.
- The fluorescence lifetime of the donor should be long enough for FRET to occur.

Evidence of FRET is seen when there is a decrease in the fluorescence and excited state lifetime of the donor while the fluorescence of the acceptor increases. Therefore, QDs are suitable as sensors through the FRET mechanism because of their broad absorption spectra, high quantum yields and long fluorescence lifetimes.

4.3 Application of Quantum Dot-based Sensors to Detect Polycyclic Aromatic Hydrocarbons

PAHs are a class of organic compounds with fused benzene rings. PAHs are of environmental concern because of their negative health effects. These compounds can be introduced into environmental water systems through several ways after wet or dry deposition from the atmosphere after their release from sources including industrial combustion activities, biomass burning, and vehicular emissions. A comprehensive review on the occurrence and typical concentration ranges of PAHs in South African water systems is provided by Chimuka et al. (2015) and is presented in Table 7.

Table 7: Typical range of PAH concentrations in run-off and river water in South Africa (Chimuka et al., 2015)

PAH	Run-off water Range (μg·L^{-1})	River water Range (μg·L^{-1})
Indene	1.3–10.1	0.8–3.3
Azulene	9.5–134	n.d.
Dibenzothiophene	0.6–67.5	0.3–9.4
Anthracene	6.7–230	6.7–53.5
Fluoranthene	3.4–251	3.6–24.2
Pyrene	7.2–2500	0.1–52.4
Mean values	4.78–532.1	2.3–28.56
	Jukskei River water[2]	**River and dam water[2]**
Naphthalene	0.025–0.145	0.022–0.239
Acenaphthene	0.036–0.239	0.053–0.407
Phenanthrene	0.119–0.197	0.053–0.616
Fluoranthene	0.046–0.201	0.021–0.890
Pyrene	0.030–0.104	0.024–0.089
Mean values	0.051–0.177	0.035–0.480

1 = Samples taken from the Thohoyandou area, Limpopo Province
2 = Samples taken from eastern, central and western areas of Johannesburg

Clearly these compounds occur at low concentrations in run-off and river water, but continuous exposure may pose health effects. Thus, routine monitoring is necessary to ensure water quality, hence cost-effective and highly sensitive and selective screening methods may provide a useful alternative to current conventional analytical techniques.

A summary of existing methods that can be used for field analysis of hydrocarbons in petroleum, which are also applicable to the analysis of PAHs and other semi-volatile organic compounds (SVOCs), is presented in Table 8. The limitations of the methods are also included as well as an indication of the cost. QD fluorescence sensors have emerged as highly sensitive probes for detecting these compounds.

Thus, in this section we review existing literature on the application of QD materials for PAH detection. PAHs generally have low solubility in water, where high molecular weight PAHs such as benzo[a]pyrene are the least soluble (0.0057 mg·L^{-1} at 25°C) and lower molecular weight naphthalene have the highest solubility (31.69 mg·L^{-1} at 25°C) (CEPA, 1994). Removal of PAHs in water systems can be achieved either by using traditional methods such as destructive oxidation with ozone or by using adsorbents such as activated carbon (Zeledón-Toruño et al., 2007).

Table 8: Summary table of field methods for petroleum hydrocarbon analysis (US EPA, 1997)

Test method	Media [1]			Analyte	Data Quality Level [2]	Analysis time	Cost per sample [3]	Skill level	Limitations
	S	SG	W						
Detector tubes		✓	✓	> 100 specific compounds	1A/1B	5 to 15 min	$8 to $27	Low	High degree of cross-reactivity
Fibre optic chemical sensors		✓	✓	Volatile organic compounds (VOCs) and SVOCs ≥ C_6	1A/1B	3 to 5 min	< $1 to $10	Low	Does not measure specific constituents
Colorimetric test kits	✓		✓	Aromatic hydrocarbons	1A/1B	10 to 20 min	$17 to $42	Low–Medium	Colours may be difficult to distinguish
Total organic vapour methods with flame ionization detector and photo-ionization detector	✓	✓	✓	Total VOCs	1A/1B	1 to 30 min	< $1 to $10	Low–Medium	Does not measure specific constituents
Turbidimetric test kit	✓			TPH of mid-range hydrocarbons (e.g., diesel fuel)	1B	15 to 20 min (25 per hour)[4]	$10 to $15	Low–Medium	Not useful for gasoline
Immunoassay Test kits	✓		✓	TEX/PAHs/ TPH	1B	30 to 45 min (5 to 8 per hour)[4]	$20 to $60	Medium	Cross-reactivity may affect interpretation
Portable infrared detectors	✓		✓	TPH of hydrocarbons to C_6 to C_{26}	2	5 to 20 min	$5 to $30	Medium	VOCs are not accurately analysed
Field GC	✓	✓	✓	Specific VOCs and SVOCs	2/3	10 to 60 min[5]	$20 to $70	Medium–High	Requires a skilled technician

1 = Soil (S), Soil-Gas (SG), Water (W)
2 = Data quality levels are discussed in further detail in the following text
3 = Includes estimation of capital costs and disposables – excludes labour
4 = When run in batches
5 = Longer times result when high quality method preparations are performed

Data Quality Levels
1A = Qualitative screening
1B = Semi-quantitative screening (magnitude estimations e.g. ppm, ppb, ppt, etc.)
2 = Quantitative delineation of specific contaminants
3 = Quantitative clean zone (regulatory monitoring, determining clean samples)

4.3.1 Monitoring methods for PAHs

The principle of using QD-based sensors is based on the interaction of QDs with PAHs whereby energy transduction can occur, resulting in either quenching or enhancement of the fluorescence emission intensity of the QDs (or PAHs). If this change in fluorescence emission intensity can be mathematically related to the concentration of the analyte PAH compounds, a sensor for that compound is thus realised.

The surface of QDs can be modified and functionalised with suitable receptors for selective recognition of specific PAHs. Supramolecules such as cyclodextrins (CDs) and calixarenes (CAs) have been used for this purpose in different studies. This is because the cavities of these supramolecules selectively form inclusion complexes with compounds of specific size, allowing for the target compound to be close enough to the QDs for energy transduction to occur. A summary of the literature on PAH detection using QDs is presented in Table 9.

Table 9: Summary of studies that used QDs as probes for PAH detection

Sensor type	Capping ligand(s)	PAH analyte	Limit of detection (LOD)	Linear range	Matrix	Reference
CdSeTe/ZnSe/ ZnS – Graphene	L-cys	Phenanthrene	1.07 nM	0–0.5 µM	–	(Adegoke & Forbes, 2016)
CdTe-Graphene	Mercaptoacetic acid	Pyrene	40.2 nM	60 nM–2 µM	Tap water River water Mineral water	(Wang et al., 2015)
CdSe/ZnS	β-CD	Anthracene	16 nM	0–10 µM	–	(Han and Li, 2008)
CdTe on TiO$_2$ Nanotubes	–	Benzo(a)pyrene	15 pM	400 nM–40 pM	–	(Yang et al., 2010)
CdSe	–	Anthracene Naphthalene	20.1 ng·mL^{-1} 105.5 ng·mL^{-1}	0.21 µM–14 µM 1.5 µM–25 µM	–	(Sehatnia et al., 2014)
CdTe	Cyclodextrins	Phenanthrene Acenaphthene	0.53 µM 0.085 µM	0.5 µM–75 µM 1 µM–25 µM	Water	(Qu & Li, 2009)
CA[4]@SiO$_2$@ CdTe CA[7]@SiO$_2$@ CdTe	Calixarene (CA[*n*], *n* = 4, 7)	Anthracene Pyrene	24.5 nM 29.4 nM	0.1–50 µM	–	(Li & Qu, 2007a)
CdSe/ZnS in sol-gel matrix	–	Anthracene Phenanthrene Pyrene		0.01–0.1 µM 0.01–0.1 µM 0.005–0.05 µM	–	(Duong et al., 2011)
CdSe on SWCNTs	–	Benzo(a)-anthracene Benzo(a)pyrene Pyrene Perylene	0.0007 µM 0.0002 µM 0.0001 µM 0.0003 µM	0.002–0.1 µM 0.0007–0.05 µM 0.0004–0.05 µM 0.001–0.05 µM	Water	(Carrillo-Carrión et al., 2009)

Li and Qu (2007a) embedded CdTe QDs inside silica spheres and thereafter decorated the spheres with CAs to form CA[n]@SiO_2@CdTe nanoparticles for selective detection of PAHs (Figure 15). This allowed for the selective detection of anthracene and pyrene using CA[4]@SiO_2@CdTe and CA[7]@ SiO_2@CdTe respectively, with corresponding detection limits of 2.45×10^{-8} M and 2.94×10^{-8} M.

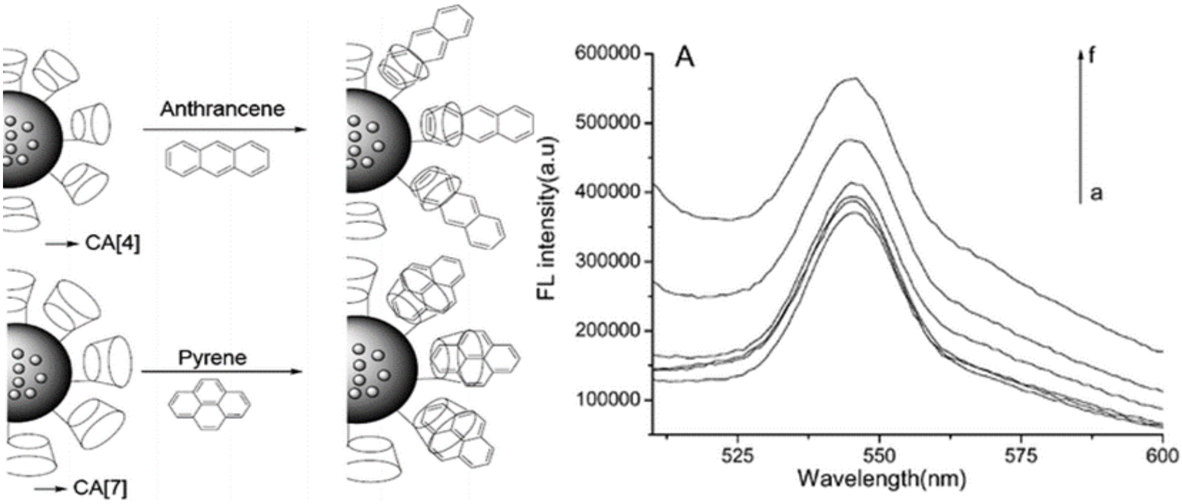


Figure 15: (a) Schematic of possible structures and interaction of CA[n]@SiO_2@CdTe sensor with anthracene and pyrene; (b) Effect of anthracene concentration on fluorescence intensity of CA[4]@SiO_2@CdTe (Li & Qu, 2007a)

CDs consist of cyclic oligosaccharides with either 6 (α-CD), 7 (β-CD) or 8 (γ-CD) glucose units linked to form a toroidal shape with a hydrophobic cavity (Szejtli, 1998). Thus, the cavities can complex with PAHs easily through hydrophobic interactions and guest-host interaction to form inclusion complexes. Because the size of the CD cavity is fixed, only compounds with an appropriate molecular size and structure can form an inclusion complex, hence selectivity is attained. Like CA, they have also been used with QDs for selective detection of PAHs. Han and Li (2008) used β-CD modified CdSe/ZnS QDs as a selective probe for anthracene detection down to 1.6×10^{-8} M.

A study by Qu and Li (2009) used β- and γ-CDs to form inclusion complexes with PAHs separately, then introduced QDs into this solution for PAH detection (Figure 16).

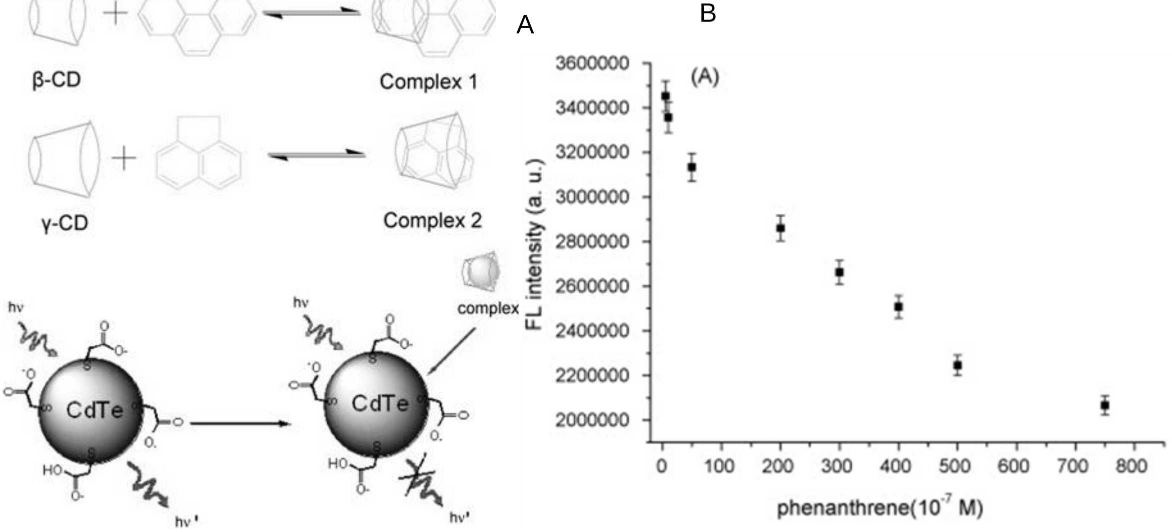


Figure 16: (a) Schematic of possible interaction of CDs with PAHs to form a complex with quenching effect upon interaction with CdTe QDs, (b) Change in QD fluorescence intensity with phenanthrene concentration (Qu & Li, 2009)

Qu and Li (2009) found that in the presence of β-CDs, only phenanthrene selectively quenched the QD fluorescence intensity, while in the presence of γ-CDs, both acenaphthene and 9,9-diflurofluore selectively quenched the intensity. This resulted in a linear decrease in fluorescence intensities. They reported detection limits of 0.53 µM and 0.085 µM for phenanthrene and acenaphthene respectively.

Graphene-conjugated QDs have also found application in PAH detection. Graphene has the advantage of a large surface area with delocalized π electrons which can adsorb and pre-concentrate other organic compounds with fused rings in their structure. Graphene-modified QDs were reported by Adegoke and Forbes (2016) who fabricated L-cys-capped CdSeTe/ZnSe/ZnS QDs, which they linked to graphene oxide (GO) to form a QD-GO nanocomposite. The probe showed PL enhancement with increasing concentration of PAH and was used to detect common PAHs including phenanthrene, anthracene, pyrene and naphthalene. Phenanthrene showed the most significant PL enhancement compared to the other PAHs and the probe showed sensitivity down to 1.07 nM. The success of the probe was attributed to strong adsorption (through π-π interaction) of the PAH molecules with the graphene sheets, which brought them closer to the QDs allowing for energy transfer, and hence detection, to occur (Figure 17).

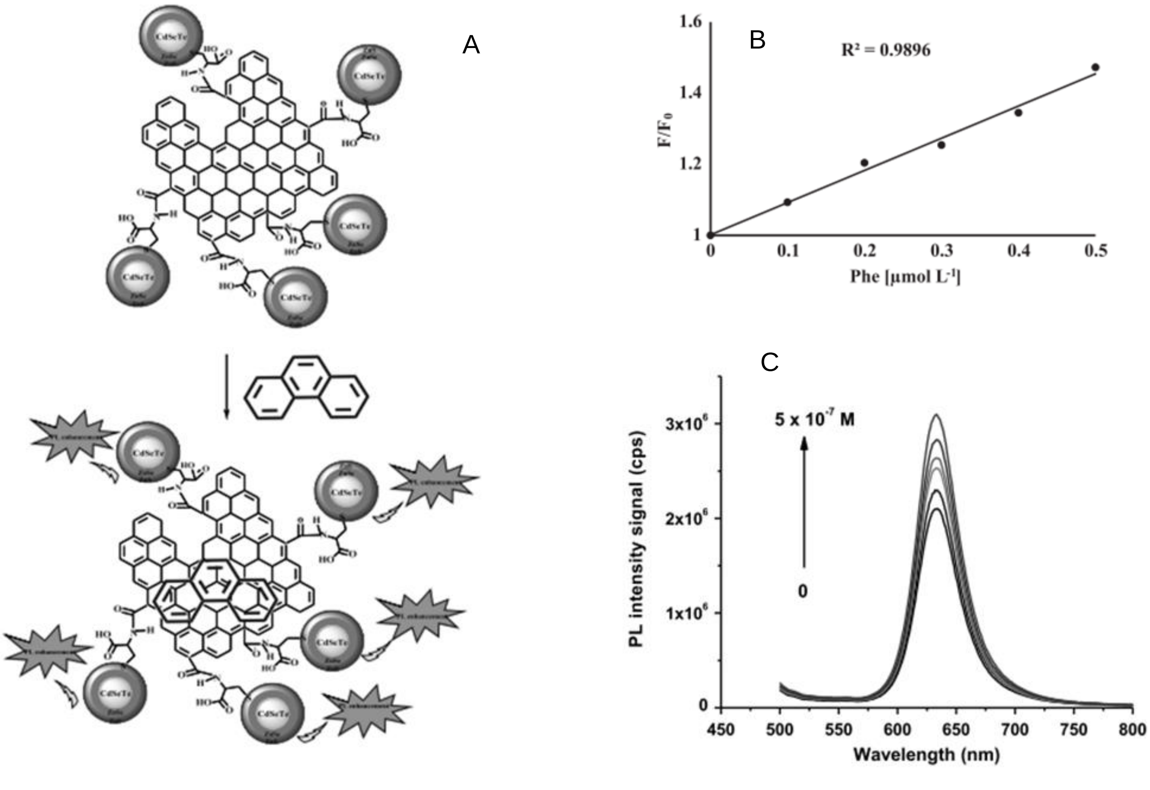


Figure 17: (a) Schematic illustration of the phenanthrene detection mechanism using a QD-GO probe, (b) Calibration curve showing linear response of the QD-GO probe with phenanthrene concentration, and (c) Fluorescence response of the probe to phenanthrene (Adegoke & Forbes, 2016)

A similar QD-graphene probe was reported by Wang et al. (2015), but their QDs only consisted of an MPA-capped CdTe core. They used the probe to detect pyrene down to 40.2 nM. Unlike in the study by Adegoke and Forbes (2016), the authors observed a quenching of the QD PL with increasing pyrene concentration. This they attributed to photo-induced electron transfer whereby upon excitation the excited electrons of the QDs are transferred to pyrene through the π–conjugated system of graphene. Both studies demonstrated the affinity of graphene towards the planar structures of PAHs, making it useful as an adsorption surface for sensor development.

Other materials that have been used to serve as adsorption surfaces to bring the PAHs closer to the QDs for improved energy transfer (FRET) include single-walled carbon nanotubes (Carrillo-Carrión et al., 2009) and TiO2 nanotubes (Yang et al., 2010).

4.4 Application of Quantum Dot-based Sensors to Detect Pesticides

South Africa is the largest user of pesticides in sub-Saharan Africa mainly due its large agriculture sector, which is driven by population and high food production demand (Dabrowski et al., 2014). This can lead to contamination of natural resources like soil and water through several pathways including run-off, spray drift deposition and leakage from the soil to groundwater. Although sunlight and microorganisms can break many pesticides down to less toxic forms, a substantial number of pesticides can persist in the environment because of their longer half-lives. The serious potential negative health effects of these pesticides to organisms are well documented in previous WRC reports (Dabrowski, 2015a; 2015b).

WWTPs can remove pesticides in water through chemical oxidation or using different adsorption technologies. A study by Ormad et al. (2008) compared the effectiveness of waste water treatment technologies to remove pesticides. They found oxidation with chlorine can remove up to 30%, while oxidation with ozone removed 70%, and a combination of these with adsorption technologies can remove up to 90% of pesticides. However, the potency of some pesticides even at low concentrations requires highly sensitive and rapid monitoring techniques.

QDs can be modified in many ways to fabricate sensors for pesticide detection, as reviewed in this section. The surface of QDs can be modified in different ways to achieve selectivity towards the target pesticides. These include:

- MIPs.
- Coupling to biological recognition elements (biosensors).
- Using guest-host interactions of supramolecular cavities, for example, CDs and CAs.

Several studies have used QDs for pesticide detection using electrochemiluminescence or electro-generated chemiluminescence (Dong et al., 2013; Du et al., 2010; Du et al., 2015; Li et al., 2015b; Liang et al., 2014; Liu et al., 2014a); however, the sensing principle of these probes will not be discussed in this report. Focus will rather be on published fluorescence-based sensors as summarised in Table 10.

Table 10: A summary of studies where QD-based probes are used for pesticide detection

	QD	Recognition element	Detection technique	Analyte(s)	LOD	Linear range	Matrix	Ref.
QD-based biosensors for pesticide detection	CdTe	AChE/ChOx	Fluorescence	Paraoxon Dichlorvos Parathion	0.00275 nM 0.00209 pM 4.82 pM	1 pM–1 µM	Apples	(Zheng et al., 2011a)
	CdTe	AChE	Fluorescence	Paraoxon Parathion	10.5 pM 4.47 pM	1 pM–1 µM	Apples, tap water, beans	(Zheng et al., 2011b)
	ZnSe:Mn^{2+}	ChOx-AChE-ACh	Fluorescence	Paraoxon	13.1 pM	48.4 pM–4.84 µM	Tap water, milk	(Gao et al., 2012)
	CdS	AChE	Fluorescence	Paraoxon	80 pM	–	Human serum	(Garai-Ibabe et al., 2014)
	MPA-CdTe	AChE and ChOx	Fluorescence	Paraoxon Parathion	4.30 pM 2.47 pM	10 pM–1.0 µM	Apples	(Xue et al., 2016)
	CdTe (QDs/PDDA)$_3$ (AChE/PDDA)$_3$	AChE	Fluorescence	Paraoxon Parathion	10 µg·L^{-1}	5–100 µg·L^{-1}	Water, apples	(Luan et al., 2016)
	CdSe/ZnS and CdSe/ZnSe	AChE and ATCh	PL	Parathion-methyl Acetamiprid	190 nM 11 200 nM	–	–	(Hai et al., 2012)
	AChE-CdTe, AChE-CdSe/ZnS AChE-CdSe/ZnSe/ ZnS	AChE	PL	Parathion-methyl Acetamiprid	1 ppb	0.05–10 ppb		(Hai et al., 2013)
	AChE-Graphene-ZnSe QDs	AChE	Fluorescence	Paraoxon	2 nM	–	–	(Nan et al., 2015)
	MPA-CdSe/ZnSe/ ZnS CdTe/CdS	AChE and ATCh	Fluorescence	Parathion-methyl	190 nM	190–3800 nM	Water	(Thi Kim Chi et al., 2012)
	MPA-CdTe	AChE and ChOx	Fluorescence	Dichlorvos	4.49 nM	Two segment: 4.49–896 nM	Apples	(Meng et al., 2013)

	QD	Recognition element	Detection technique	Analyte(s)	LOD	Linear range	Matrix	Ref.
						2240–6780 nM		
	SiQDs-AChE-ChOx	AChE and ChOx	PL	Carbaryl Parathion Diazinon Phorate	7.25×10^{-9} g·L^{-1} 3.25×10^{-8} g·L^{-1} 6.76×10^{-8} g·L^{-1} 1.9×10^{-7} g·L^{-1}	7.49 ng·L^{-1}–0.749 mg·L^{-1}	Apples, tomatoes, cucumbers	(Yi et al., 2013)
	CQDs	AChE and ChOx	PL	Carbaryl	0.0268 nM	0.0313–31.3 nM	Apples	(Li et al., 2016)
	Q-QDs	AChE	Fluorescence	Dichlorvos	0.019 nM	0.050 nM–100 nM	Fruit juice	(Hou et al., 2016)
	TGA-CdTe	AChE	Fluorescence	Monocrotophos	32.0 nM	89.5–6090 nM	Aqueous solutions	(Sun et al., 2011)
	TGA-CdSe/Chitosan films	OPH	PL	Paraoxon	1000 nM	–	–	(Constantine et al., 2003)
	CdSe/ZnS	OPH	PL	Paraoxon	10 nM	Non-linear	Water	(Ji et al., 2005)
	MPA-CdTe-CTAB	OPH	Fluorescence	Parathion-methyl	61.8 nM	95–11000 nM	Tap water, rice	(Yan et al., 2015b)
	MPA-CuInS$_2$	OPH	Fluorescence	Parathion-methyl	60 nM	100–38 000 nM	Tap water, river water, rice, bananas	(Yan et al., 2015c)
	MPA-CdTe/IgG	Anti-2,4-D antibody (IgG) ELISA	Fluoroimmunoassay	2,4-dichlorophenoxyacetic acid	1.13 nM	1130–4520 nM	–	(Vinayaka et al., 2009)
	QDs 530 goat anti-mouse IgG conjugate (QDs-Ab$_2$)	cFLISA ELISA	Fluorescence	Chlorpyrifos	24 nM 46 nM	43–586 nM	Drinking water	(Chen et al., 2010)
	ZnS:Mn^{2+}	DNA aptamer	Fluorescence	Acetamiprid	0.7 nM	0–15 nM	Water, cabbage leaves	(Lin et al., 2016)

	QD	Recognition element	Detection technique	Analyte(s)	LOD	Linear range	Matrix	Ref.
MIP-based sensors for pesticide detection	CdTe-AuNPs	DNA aptamer	Fluorescence	Acetamiprid	7.29 nM	50–1 000 nM	Lettuce, pakchoi, cauliflower, pamphrey, Chinese cabbage, celery	(Guo et al., 2016)
	IgG-CDs	IgG	Fluorescence	Glyphosate	8 ng·mL^{-1}	0.01–80 µg·mL^{-1}	River water, soil samples	(Wang et al., 2016a)
	CDs-AgNPs	AChE	Fluorometric Colorimetric	Carbaryl	0.006 µg·L^{-1} 0.007 µg·L^{-1}	1 × 10^{-8} –1 × 10^{-4} g·L^{-1}	Tap water, apple juice	(Zhao et al., 2016)
	Cys-CdTe/ATP	ACP	Fluorescence	Parathion-methyl	1.2 ng·mL^{-1}	0.001–5.0 g·mL^{-1}	Water	(Wang et al., 2016b)
	C-dots/ZrO_2-GCE		Electrochemical (CV)	Methyl parathion	0.056 ng·mL^{-1}	0.2 ng·mL^{-1} to 48 ng·mL^{-1}	Rice	(ReddyPrasad et al., 2015)
	ZnS:Mn^{2+}	MIP	Fluorescence	Pentachlorophenol	86 nM	200–3 900	River water	(Wang et al., 2009b)
	ZnS:Mn^{2+}	MIP	Fluorescence	Diazinon	164 nM	164–2 000	Tap water	(Zhao et al., 2012)
	CdSe@SiO_2@MIP	MIP	Fluorescence	λ-cyhalothrin	80.03 nM	100–1 000 000 nM	Water	(Li et al., 2010)
	CdTe-SiO_2-MIPs	MIP	Fluorescence	Deltamethrin	31 700 nM	1000–69000 nM	Fruit and vegetable samples	(Ge et al., 2011)
	ZnS:Mn^{2+}	MIP	Fluorescence	Nicosulfuron	1.1 nM	12–6 000 nM	Water	(Ren & Chen, 2015a)
	ZnS:Mn^{2+}	MIP	Fluorescence	Cyphenothrin	9.0 nM	100–80 000 nM	Water	(Ren & Chen, 2015b)
	ZnS:Mn^{2+}	MIP	Fluorescence	Chlorpyrifos	17 nM	300–60 000 nM	Water	(Ren et al., 2015)

	QD	Recognition element	Detection technique	Analyte(s)	LOD	Linear range	Matrix	Ref.
Based on other functional materials	Magnetic silica beads/graphene QDs/MIP (mSGP)	MIP	Fluorescence	Tributyltin	146.7 nM	0–48 000 nM	Seawater	(Walia & Acharya, 2014)
	CdSe/ZnS	MIP	Fluorescence	Carbaryl	147 nM	497 –398 000 nM	Rice, Chinese cabbage	(Zhang et al., 2014)
	Carbon dots	MIP	Fluorescence	Dimethoate	0.0183 nM	0.6–34 nM	Chinese cabbage, broccoli, Cucumber	(Li et al., 2015a)
	OVDAC/CdTe	MIP	Fluorescence	λ-cyhalothrin	30 nM	100–16 000 nM		(Wei et al., 2016)
	CdTe	MIP	Fluorescence	Parathion	218 nM	50–1 000 000 nM	Water	(Tang & Xiang, 2016)
	ZnS:Mn^{2+}	MIP-Fe_3O_4	Fluorescence	Pentachlorophenol	500 nM	0–30 000 nM	Water	(Yang et al., 2012)
	CdTe/CdS		Laser Induced Fluorescence	Mevinphos Methidathion Diazinon Phosalone	0.16 mg kg^{-1} 0.05 mg kg^{-1} 0.18 mg kg^{-1} 0.12 mg kg^{-1}	0.2–28.0 mg kg^{-1} 0.1–20.0 mg kg^{-1} 0.3–18.0 mg kg^{-1} 0.2–30.0 mg kg^{-1}	Tomatoes	(Chen & Fung, 2010)
	C[4]/SiO_2/CdTe	Calix[4]arene	Fluorescence	Methomyl	80 nM	0.1–50 µM	Water	(Li & Qu, 2007b)
	C[6]/SiO_2/CdTe	Calix[6]arene	Fluorescence	Glyphosate	0.0725 nM	1.0–25.0 nM	Water	(Li et al., 2012b)
	TGA-CdTe	p-sulfonatocalix[4]arene	Luminescence	Fenamithion Acetamiprid	12 nM 34 nM	0–10^{-4} M 0–10^{-3} M	Garlic samples, Aqueous solution	(Qu et al., 2009)
	ZnCdSe films	–	Fluorescence	Carbaryl	< 12.4 nM	12.4–12400 nM	Water	(Bakar et al., 2011)
	Graphene-CdS	–	Electrochemiluminescence	Pentachlorophenol	0.0113 nM	37 pM–1.9 µM	Tap water, River water	(Liu et al., 2014a)

	QD	Recognition element	Detection technique	Analyte(s)	LOD	Linear range	Matrix	Ref.
	GSH-CdS	–	Fluorescence	Dicofol	55 ±11 ppb	3.32–62.5 µM	Methanol	(Walia & Acharya, 2014)
	RF-CdTe@SiO$_2$-AuNPs	Trypsin/ protamine	Fluorescence	Parathion-methyl	0.0684 nM	0.15 nM–1500 nM	Tap water, milk, rice	(Yan et al., 2015a)
	MPA-CdTe	–	Fluorescence	Chlorpyrifos	0.1 nM	0.1–10000 nM	Apples	(Zhang et al., 2010a)
	TGA-CdTe/CS-AuNPs	–	Fluorescence	Glyphosate	9.8 ng kg^{-1}	0.02–2.0 µg·kg^{-1}	Apples	(Guo et al., 2014)
	CdTe/CdS	–	Fluorescence	Paraquat	6.39 nM	9.90–1500 nM	Rice, wheat flour, lettuce leaves, waste water	(Bian et al., 2010)
	(3-MPA)-CdSe/ZnS	–	Fluorescence	Paraquat	0.0117 nM	0.039–19 nM	Water	(Durán et al., 2013)
	Double QDs (ZnCdSe and CdSe)	–	Fluorescence	Paraquat Dursban Dipterex Methyl thiophanate Cartap	20 nM	2 ×10^{-8}–5 µM 5 ×10^{-8}–3 µM 2 ×10^{-8}–5 µM 5 ×10^{-8}–5 µM 5 ×10^{-8}–5 µM	Waste water, Tea,	(Fan et al., 2016)
	CDs/AgNPs	–	Fluorescence	Glyphosate	12 ng·mL^{-1}	0.025–25 ug·mL^{-1}	Rice, millet, wheat flour, and maize flour	(Wang et al., 2016c)

<u>Abbreviations</u>: GSH – Glutathione; **RF** – Ratiometric fluorescent; **TGA** – Thioglycolic acid; **CDs** – Carbon dots; **OVDAC** – Octadecyl-4-vinylbenzyl-dimethyl-ammonium chloride; **ATP** – Adenosine triphosphate**, ACP** – Acid phosphate; **Cys** – cysteine; **cFLISA** – Competitive fluorescence-linked immunosorbent assay; **CQDs** – Carbon QDs; **CTAB** – Cetyltrimethylammonium bromide; **ELISA** – Enzyme-linked immunosorbent assay; **IgG** – Glyphosate antibody; **MPA** – Mercaptopropionic acid; **Q-QDs** – Quaternized carbon dots; **TGA** – Thioglycolic acid; **PDDA** – Polydiallyldimethylammonium chloride; **OPH** – Organophosphorus hydrolase; **GCE** – Glassy carbon electrode

4.4.1 Monitoring methods for pesticides

4.4.1.1 Doped QDs for pesticide detection

Doping involves incorporating atoms [usually transition metals like manganese (Mn^{2+})] into the lattice structure of a material to avail the desired properties. Doped QDs retain almost all the properties of undoped QDs, and may have added advantages of longer emission lifetimes and lower cytotoxicity (Wu & Yan, 2013). For example, a study by Cao et al. (2014) showed that the Mn^{2+}-doped CuInS/ZnS QDs had extremely long radiative excited state lifetimes (3.78 ms) compared to those that were not doped (401 ns). Similarly, Subha et al. (2013) reported 0.35 ms lifetimes for Mn^{2+}-doped ZnS QDs, which was 4–5 orders higher than other semiconductor QDs.

Many studies used ZnS:Mn^{2+} QDs to design fluorescence sensors for pesticides including pentachlorophenol (Wang et al., 2009b; Yang et al., 2012), cyphenothrin (Ren & Chen, 2015b) and acetamiprid (Lin et al., 2016). The doped QDs may also be coupled to a recognition element like aptamers (Lin et al., 2016), enzymes (Gao et al., 2012) or MIPs (Ren & Chen, 2015a; Ren & Chen, 2015b; Ren et al., 2015; Wang et al., 2009b; Zhao et al., 2012) to achieve selectivity towards target pesticides (discussed in sections to follow).

4.4.1.2 Silica nanospheres

Sensor materials based on QDs incorporated into silica (QD-Si) nanoparticles have been used by different research groups to design fluorescence sensors used to detect different pesticides such as λ-cyhalothrin, methomyl, glyphosate and parathion-methyl in water (Li et al., 2010; Li et al., 2012b; Li & Qu, 2007b; Yan et al., 2015a). The preparation of QD-Si nanoparticles can be achieved via various strategies, where reverse microemulsion is the most commonly used approach (Goftman et al., 2016; Wang et al., 2009a; Wang et al., 2013b; Yang et al., 2007). Preparation of spheres doped with QDs can be achieved directly through co-condensation of the silica precursors (Bottini et al., 2007) and, depending on use, the surface of the sphere can be decorated with suitable ligands or receptor molecules.

The advantage of encapsulating QDs into silica spheres is that it improves their chemical stability thereby reducing leaching and potential toxicity of the QDs. It also has been found to reduce photobleaching without affecting the photophysical properties of the QDs (Hu & Gao, 2010; Ma et al., 2015a). The abundance of silicon on earth makes these materials affordable and thus potentially useful for preparing low-cost sensing devices. Another advantage of using silica nanoparticles is that they are chemically inert and optically transparent, which make them suitable for fluorescence sensing applications in various chemical media (Bapat et al., 2016). Furthermore, the silica materials are amenable to surface modifications and this makes it possible to functionalise them with receptors like calix[n]arenes (Li & Qu, 2007b; Li et al., 2012b), MIPs (Ge et al., 2011; Li et al., 2010), or with graphene sheets (Zor et al., 2015) to offer selectivity towards the target pesticide analyte. Such modified QDs and their applications are discussed the following sections.

4.4.1.3 Macrocyclic/supramolecular modified QDs

Guest-host interactions by means of macrocyclic molecules with suitable cavities can also be used in conjunction with QDs as recognition elements for pesticide detection. Calix[n]arene supramolecules, for example, have hydrophobic cavities that can be used as receptors for different target pesticides. It is possible to achieve some selectivity by tuning and/or selecting the appropriate size of the calix[n]arene. As such they have been used to functionalise the surface of QDs in different studies to develop probes for pesticide detection.

Li and Qu (2007b) fabricated silica spheres that were embedded with CdTe QDs in their core and were coated with calix[4]arene on the surface for pesticide detection. The probe showed fluorescence enhancement with increase in methomyl concentration and allowed for its detection down to 80 nM. Their probe also showed good selectivity in the presence of other pesticides, namely parathion-methyl, fenamithion, optunal, and acetamiprid.

Qu et al. (2009) functionalised the surface of CdTe QDs with p-sulfonatocalix[4]arene to produce a florescent sensor to detect fenamithion and acetamiprid down to 12 nM and 34 nM, respectively. They found that the p-sulfonatocalix[4]arene offered selectivity towards acetamiprid compared to other pesticides (parathion-methyl, methomyl, fenamithion, and optunal).

Li et al. (2012b) used calix[6]arene to develop a fluorescence sensor to detect the pesticide glyphosate. Here, CdTe QDs embedded in silica spheres that were then functionalised with calix[6]arene were employed (Figure 18). The probe was selective for glyphosate in the presence of other pesticides (chlorpyrifos, cyromazine, carbaryl, imidacloprid, methiocarb) and allowed for its detection down to 0.0725 nM, which they found to be lower than conventional HPLC and MS techniques at that time.

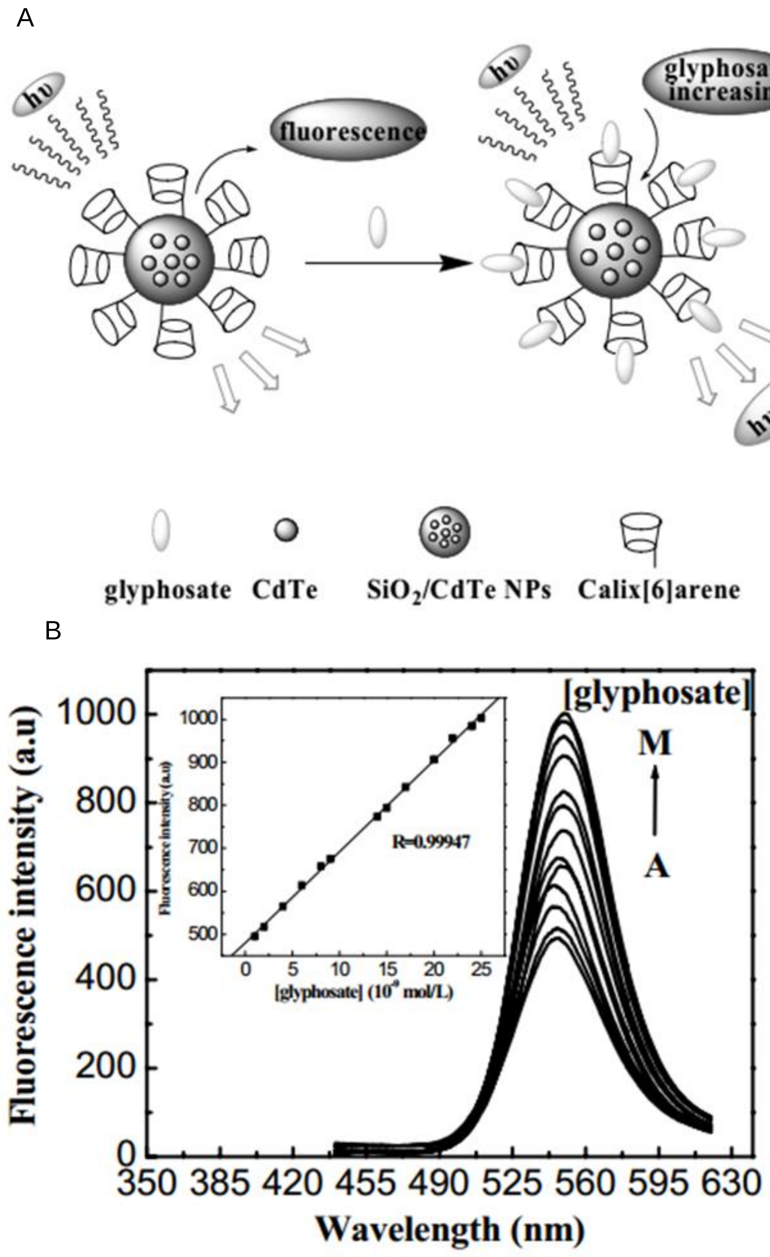


Figure 18: (a) Fluorescence sensor for ultra-sensitive detection of glyphosate; (b) Response of the sensor to increasing glyphosate concentration (Li et al., 2012b)

Although this strategy shows promising results, the binding affinity of supramolecular host molecules can be affected by a range of factors including the binding mechanism, the medium pH, stereo-electronic effects and allosteric effects (Schneider, 2015). Thus, a probe based on such receptors could suffer serious limitations in terms of repeatability, making it difficult to design standard methods based on this technology.

4.4.1.4 MIP-coated QDs for fluorescence detection of pesticides

QDs modified with MIPs as receptors or recognition elements can be used to develop probes for pesticide detection in various media. Advantages of such materials are that they are cheap, have high mechanical and chemical stability and can be reusable. They have therefore found application in several QD-based fluorescence sensors where they offer selectivity for different target pesticide analytes (Ge et al., 2011; Li et al., 2010; Li et al., 2015a; Ren & Chen, 2015a; Ren & Chen, 2015b; Tang & Xiang, 2016; Walia & Acharya, 2014; Wang et al., 2009b; Wei et al., 2016; Yang et al., 2012; Zhang et al., 2015; Zhao et al., 2012). Table 12 includes a summary of pesticides that have been studied with MIP QD fluorescence-based sensors.

By encoring a suitably tailored MIP on the QD surface, it is possible to achieve selectivity for different target pesticides even when using the same QD materials as signal transducers. For example, recent studies by Ren and Chen (2015b) used MIP-coated manganese-doped ZnS QDs for selective detection of the pesticide cyphenothrin (Figure 19).

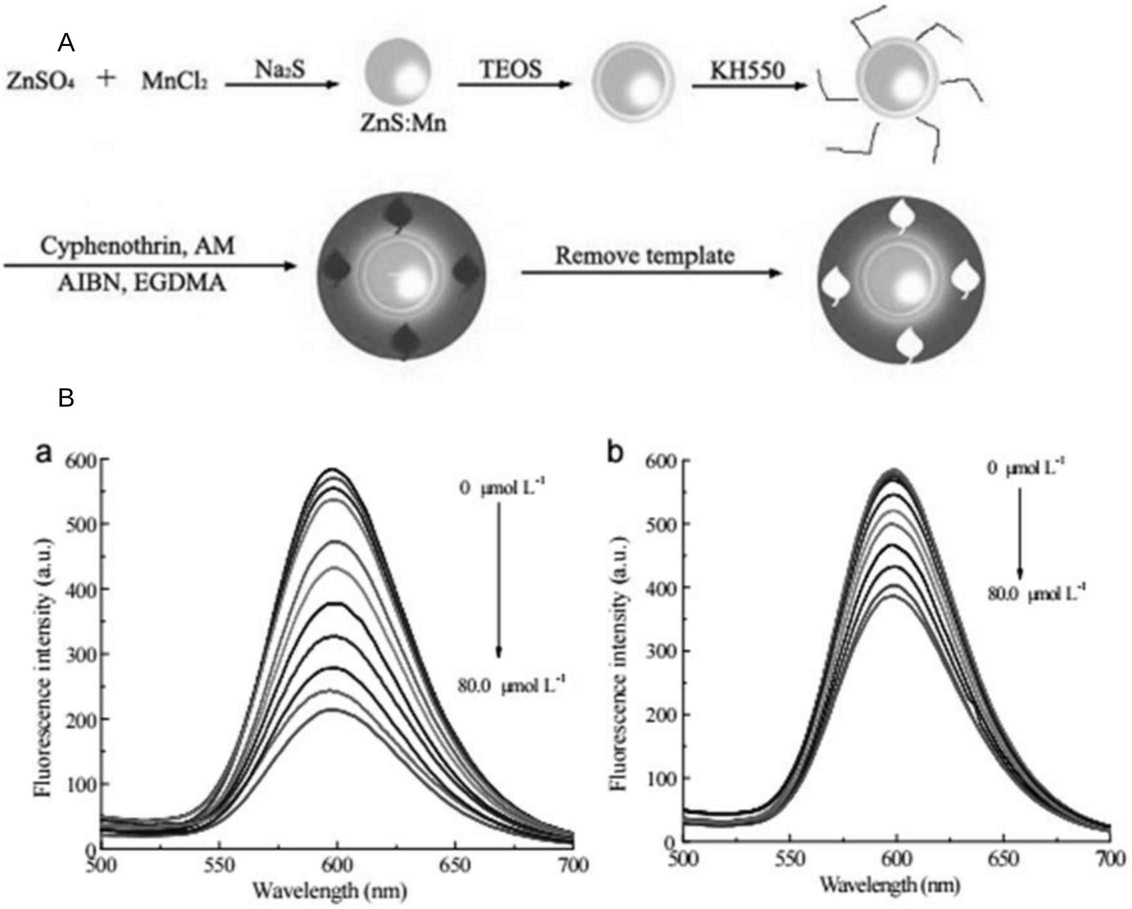


Figure 19: a) Preparation of MIP-coated ZnS:Mn QDs for detection of cyphenothrin (TEOS: Tetraethoxysilane, KH550: silane coupling agent, AM: functional monomer, EGDMA: cross-linking agent); (b) (left) Fluorescence intensity of the probe decreased with increasing cyphenothrin concentration, (right) fluorescence of NIP-coated QDs with increasing cyphenothrin concentration (Ren & Chen, 2015b)

The selectivity towards cyphenothrin was attributed to the size, shape, and functionality of the template and it allowed for detection down to 9.0 nM in water. With the same QD and by simply changing the template for the MIP, other sensors were prepared for detecting the herbicide nicosulfuron in water down to 1.1 nM (Ren & Chen, 2015a) and the insecticide chlorpyrifos in water with limits of detection down to 17 nM (Ren et al., 2015).

MIPs can also be used as a functional coating around silica-embedded QDs in pesticide sensing. For example, Li et al. (2010) prepared CdSe QDs embedded in silica spheres, which they modified with MIPs for selective trace detection of lambda-cyhalothrin in water (Figure 20). Energy transduction between the CdSe@SiO$_2$@MIP particles and the pesticide quenched the luminescence intensity of the chemophores linearly as described by the Stern–Volmer type equation:

$$\frac{l_{max}}{l} = 1 + K_{SV}[S]$$

Where I is fluorescence intensity of CdSe@SiO$_2$@MIP at a given pesticide concentration, and I_{max} is the intensity in a pesticide-free solution. K_{SV} is the Stern–Volmer quenching constant and *[S]* is the pesticide concentration. Thus, the authors showed the probe could selectively detect lambda-cyhalothrin down to 3.6 µg·L^{-1}.

In another study, Ge et al. (2011) modified CdTe-doped silica spheres with MIP to form CdTe-SiO$_2$-MIP composites for selective detection of deltamethrin in fruit and vegetable matrices. Just as with the probe by Li et al. (2010), the fluorescence intensity of the probe was quenched with increasing deltamethrin concentration. The intensity could also be related to the deltamethrin concentration using the Stern–Volmer equation, allowing for its detection down to 0.16 µg·mL^{-1}.

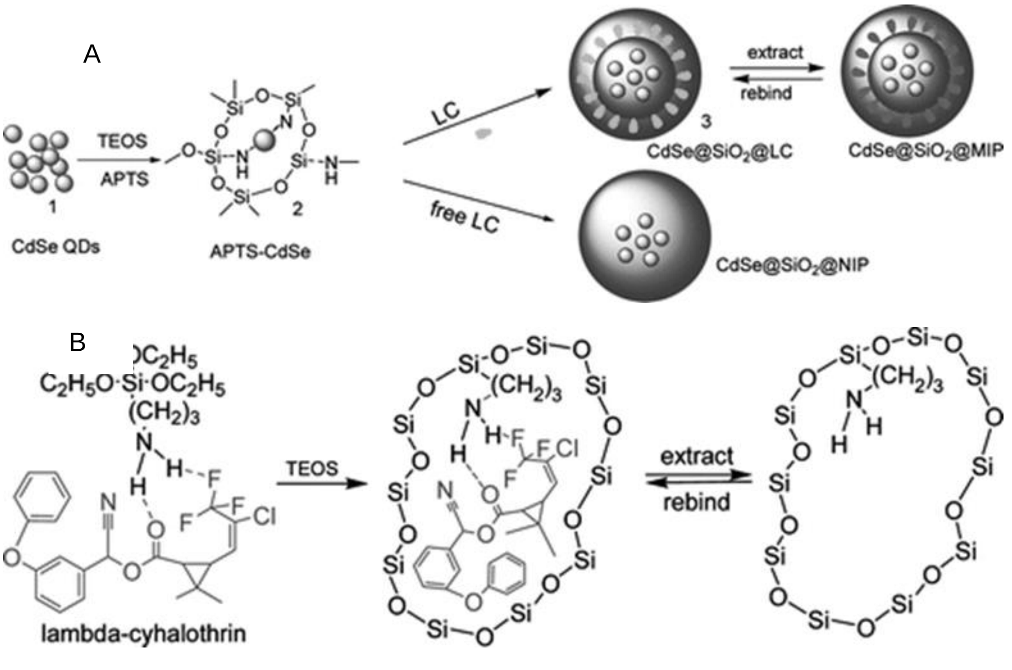


Figure 20: (a) Schematic illustration showing the molecular imprinting process for λ-cyhalothrin (LC) imprinted silica nanospheres embedded CdSe QDs (APTS: 3-aminopropyltriethoxysilane, TEOS: tetraethoxysilane); (b) Schematic illustration for the molecular imprinting mechanism of LC in a silica matrix through the hydrogen bond reaction (Li et al., 2010)

Although MIP-based sensors have been successfully employed, they also have some limitations in terms of preparations, with the main challenge being the extraction of template molecules from the polymers. This is often a tedious process that require a significant amount of solvents and may lead to distortion of some of the cavities. Overcoming this challenge is crucial to develop standardised probes and to pave a way for their commercialisation.

4.4.1.5 Carbon-based QD probes for pesticide detection

Carbon QDs are a class of fluorescent nanoparticles that include GQDs, polymer dots, and carbon nanodots, and have found several applications in florescence sensing (Baptista et al., 2015; Dong et al., 2015; Dong et al., 2016; Kong et al., 2015; Roy et al., 2015). These types of QDs offer unique fluorescence properties and have the advantage of low toxicity and excellent biocompatibility compared to semiconductor QDs (Zhu et al., 2015). Carbon QDs are an emerging alternative to semiconductor sensors and some authors have used them in designing pesticide probes.

A study by Nan et al. (2015) used GQDs in conjunction with acetylcholinesterase (AChE) enzyme for sensitive detection of paraoxon, a highly toxic organophosphate pesticide, down to 0.2 nM. Liu et al. (2014a) used GQDs linked to CdS QDs to form GQD-CdS nanocrystals for detecting pentachlorophenol, an organochlorine pesticide, in water samples. The GQDs were used as signal amplification material; thus, low detection limits (3 pg·mL^{-1}) were obtained. In recent studies, carbon dots have also been used to detect pesticides like parathion-methyl (Hou et al., 2015), dimethoate (Li et al., 2015a) dichlorvos and carbaryl (Li et al., 2016) at low detection limits (Table 10). Like other QDs, these can also be used with enzymes or MIPs to achieve selectivity for target pesticides. Zor et al. (2015) also used a graphene-based QD composite material (Figure 21) that could be used to detect the pesticide tributyltin. Again, the graphene played a role in water dispersibility of the material.

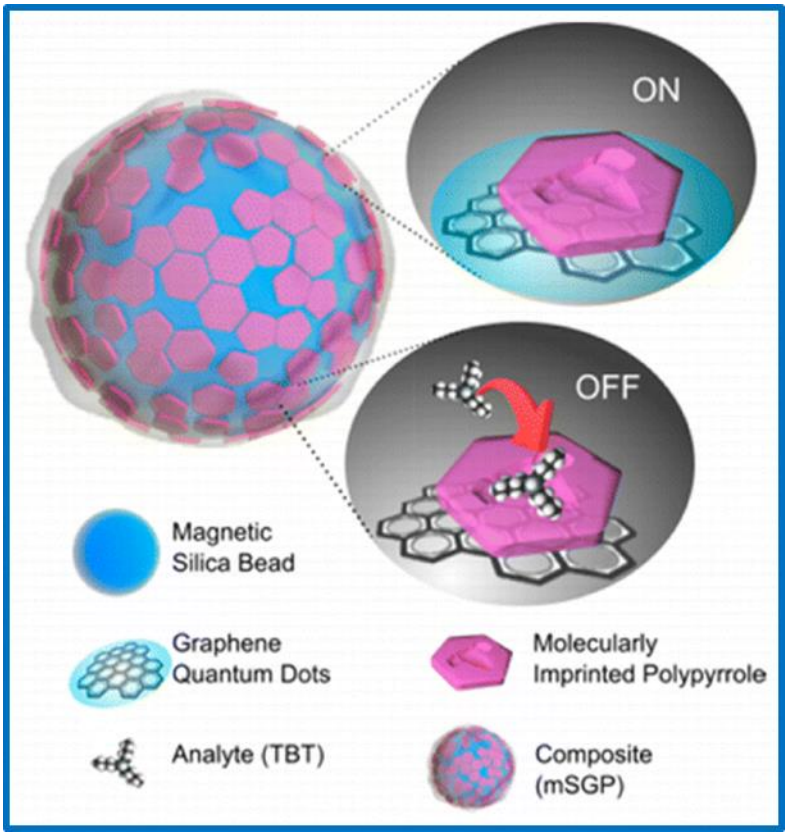


Figure 21: Schematic representation of GQD florescence sensor for detecting tributyltin. A molecularly imprinted polymer is used to offer selectivity (Zor et al., 2015)

A disadvantage of carbon dots is their poor quantum yields compared to semiconductor QDs. However, current research shows that it is possible to produce high quantum yield carbon dots through, for example, co-doping the carbon dots with nitrogen and sulphide atoms (Baptista et al., 2015; Du & Guo, 2016; Li et al., 2016; Sun et al., 2015).

4.4.1.6 Quantum dot-based fluorescence biosensors for pesticide detection

QDs have been used in conjunction with biological elements to form biosensors used for pesticide detection and quantification. A biosensor can be described as a probe that consists of a signal transducer (e.g. QDs or a fluorophore) linked to a biological recognition element, which could be an enzyme, cell, antibody, or deoxyribonucleic acid (DNA) (Kumar et al., 2015; Sassolas et al., 2012). For instance, DNA aptamers that selectively bind to acetamiprid have recently been used to detect this insecticide with QDs as signal reporters (Guo et al., 2016; Lin et al., 2016). However, screening and selecting the appropriate aptamer for the target pesticide can be challenging, which may limit their application as recognition elements.

QD enzyme biosensors have been widely explored for pesticide detection, particularly for organophosphorus pesticides. Enzymes are particularly attractive as recognition elements in QD biosensors because of their specificity towards target pesticides.

One commonly used enzyme for these probes is AChE – an enzyme found in nervous systems. Reviews on the use of AChE as a recognition element in different pesticide detection techniques using various types of nanomaterials have been published (Dhull et al., 2013; Llorent-Martínez et al., 2011; Periasamy et al., 2009; Pundir & Chauhan, 2012; Xia et al., 2015). Here we focus on techniques where QDs are used as transducers for fluorescence detection.

AChE hydrolyses the neurotransmitters acetylthiocholine and acetylcholine to thiocholine and acetylcholine, respectively (Equations 1–2). If the sensor is coupled with the choline oxidase (ChOx) enzyme, then choline can be oxidised to generate hydrogen peroxide (H_2O_2) (Equation 3), which can quench the fluorescence intensity of QDs (Yi et al., 2013).

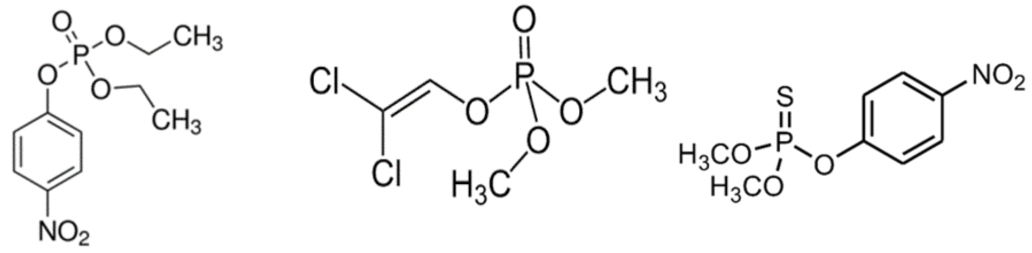
$$\text{acetylthiocholine} + H_2O \xrightarrow{\text{AChE}} \text{thiocholine} + \text{acetic acid} \qquad \text{Eq. 1}$$

$$\text{acetylcholine} + H_2O \xrightarrow{\text{AChE}} \text{choline} + \text{acetate} \qquad \text{Eq. 2}$$

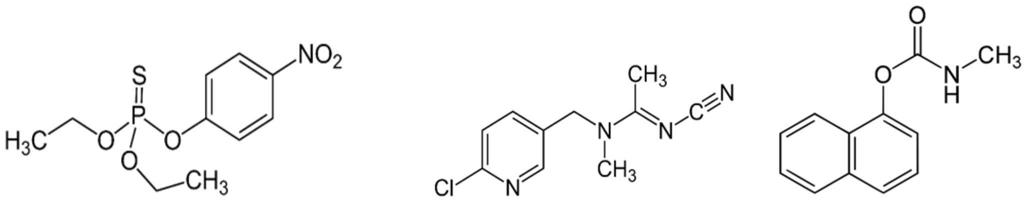
$$\text{choline} + O_2 \xrightarrow{\text{ChOx}} \text{betaine aldehyde} + H_2O_2 \qquad \text{Eq. 3}$$

Trace amounts of organophosphate pesticides and carbamate type pesticides can inhibit the activity of the ChOx enzyme (the mechanism that leads to the paralysis and death of insects), which leads to an increase in PL intensity of the conjugated QDs. The ability of the enzyme to recognise pesticide molecules has allowed for the development of QD-based sensors for these target analytes. Like other previously discussed sensors, enzyme-based sensors can also be conjugated with other support platforms like graphene (Dong et al., 2013; Nan et al., 2015) and multi-walled carbon nanotubes (Du et al., 2010; Lin et al., 2016) to improve their sensitivity.

Typical pesticide target analytes of QD/AChE biosensors are shown in Figure 22. The structurally similar pesticides parathion-methyl, paraoxon, and parathion have been extensively and successfully targeted using this type of sensor possibly due to similar interactions that they have with the enzyme.



Paraxon Dichlorvos Parathion-methyl



Parathion Acetamiprid Carbaryl

Figure 22: Chemical structures of pesticides commonly used as targets in QD-AChE based fluorescence biosensors

Yi et al. (2013) used silicon QDs (SiQDs), AChE and ChOx to fabricate a biosensor for detecting pesticides (carbaryl, parathion, diazinon, phorate) in food samples (Figure 23). They found the inhibiting effect of these pesticides on the enzyme to be linearly proportional to the logarithm of the pesticide concentration. The accuracy of the probe was comparable to traditional HPLC methods with the added advantage of being highly sensitive, simple and rapid.

Another enzyme used in conjunction with QDs for pesticide detection is organophosphorus hydrolase (OPH) that hydrolyses a variety of organophosphate pesticides to produce harmless products like *p*-nitrophenol and diethyl phosphate. Depending on the type of QD, both these products can interact with the QDs causing either an increase or decrease in their fluorescence intensity thereby allowing for detection. This enzyme has been used in QD sensors to detect the pesticides paraoxon (Constantine et al., 2003; Ji et al., 2005) and parathion-methyl (Yan et al., 2015b; Yan et al., 2015c) in matrices like river water.

Yan et al. (2015b) used CdTe QDs in cetyltrimethylammonium bromide (CTAB) and OPH to make a biosensor for parathion-methyl detection. Presence of the pesticide results in the quenching of the PL intensity of the QDs because *p*-nitrophenol (product of hydrolysis by OPH) is electron deficient and is therefore adsorbed on the alkyl chains of CTAB through hydrophobic interactions, thereby quenching the PL intensity of the QDs. This quenching effect was proportional to the parathion-methyl concentration in the 25–3000 ng·mL^{-1} range and allowed for detection down to 18 ng·mL^{-1} in tap water.

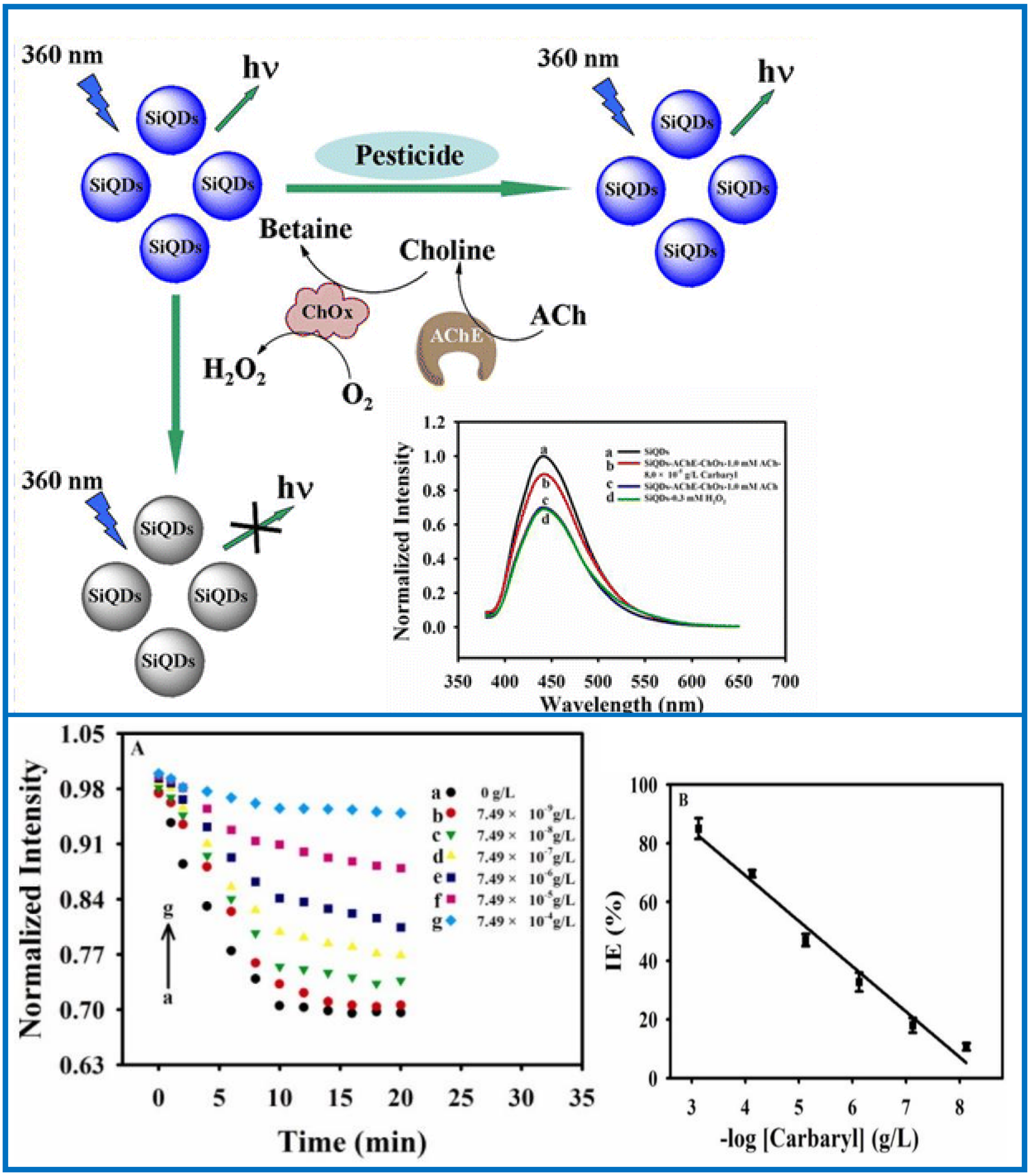


Figure 23: a) Schematic illustration of the working principle of the AChE-based biosensor for pesticide detection; (b) Incubation time dependence of fluorescence intensity of SiQDs-AChE-ChO probe at different concentrations of carbaryl; (c) Inhibition efficiency versus log of carbaryl concentration under optimised conditions (Yi et al., 2013)

In a different study, Yan et al. (2015c) made a biosensor consisting of $CuInS_2$ QDs, lead ions (Pb^{2+}) and the OPH enzyme, which was also used for detecting parathion-methyl. The fluorescence intensity of the probe was quenched by Pb^{2+} ions. Instead of *p*-nitrophenol, this probe used the other OPA hydrolysis product, dimethyl-thiophosphoric acid, which bonded to Pb^{2+} ions resulting in an increase in PL intensity with pesticide concentration (Figure 24).

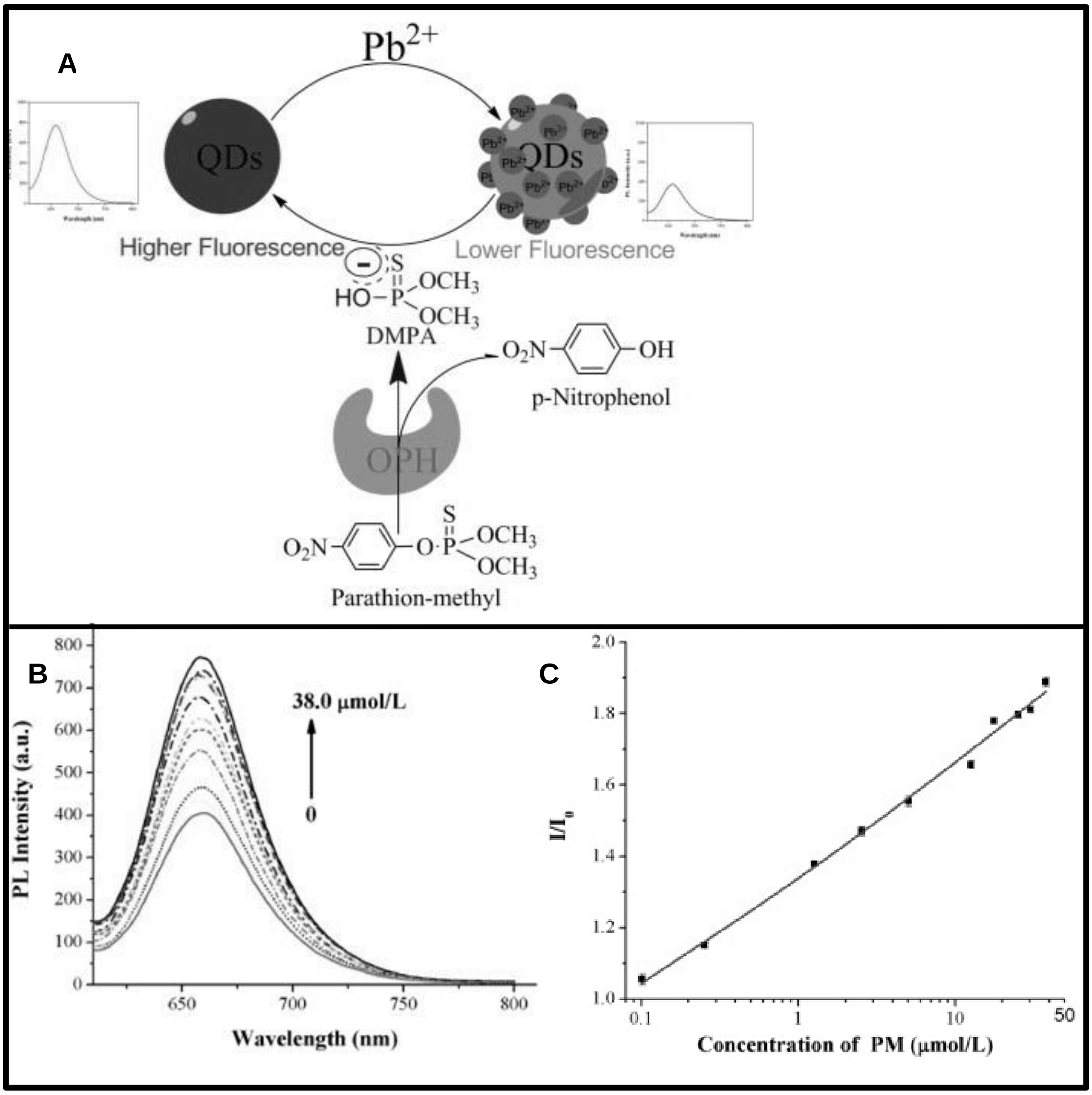


Figure 24: (a) Schematic diagram illustrating the working principle of the CuInS$_2$ QD biosensor for parathion-methyl (PM) detection (Abbreviations: DMPA: dimethyl-thiophosphoric acid, OPH: Organophosphorus hydrolase); (b) Response of the sensor to increasing concentration of parathion-methyl; (c) Plot of fluorescence intensity ratios of the CuInS$_2$ sensor versus the logarithm of concentration of PM. I and I$_0$ were the fluorescence intensity of CuInS$_2$ QDs complex in the presence and absence of PM, respectively (Yan et al., 2015c)

4.4.1.7 QDs for organochlorine pesticides

Application of QDs for detecting organochlorine pesticides has been rather limited. Walia and Acharya (2014) used GSH-coated CdS (GSH-CdS) QDs for selective detection of dicofol, an organochlorine pesticide, in the presence of other pesticides (dimethoate, chlorpyrifos, imidacloprid). Control experiments suggested that the chloride groups in dicofol (Figure 25) interact with the $-NH_2$ and $-COOH$ groups of GSH, leading to an increase in fluorescence of the GSH-CdS QDs. They were able to detect dicofol down to 55 ± 11 ppb.

Liu et al. (2011) developed a fluorescence sensor for atrazine detection. Fluorescent signal transduction for their probe was based on a dye (zinc protoporphyrin) rather than on QDs. There is therefore a research gap in developing QD-based sensors for organochlorine pesticides.

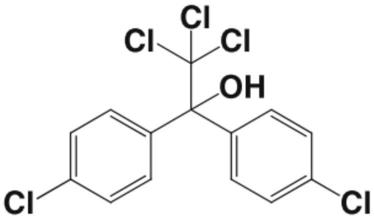


Figure 25: Chemical structure of the dicofol

4.5 Application of Nanoparticles to Determine Triclosan in Water

Triclosan, 5-chloro-2-(2,4-dichlorophenoxy) phenol (Figure 26) with the commercial name Irgasan DP300, has been used in a variety of consumer products (Bhargava & Leonard, 1996; Jones et al., 2000).

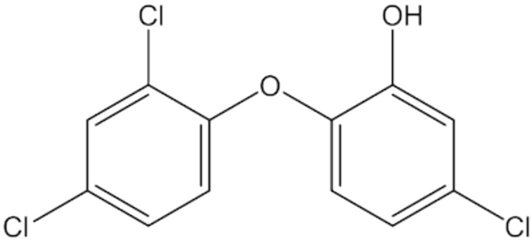


Figure 26: Chemical structure of triclosan

Triclosan is a fairly small molecule with a molecular weight of 289.54 $g \cdot mol^{-1}$ and a diameter of ±7.4 Å (Rossner et al., 2009). It is a white solid at standard temperature and pressure, with a boiling point in the range of 280–290°C and a melting point in the range of 56–58°C. The solubility of triclosan in water is $< 10^{-6}$ $g \cdot mL^{-1}$ (Du Preez & Yang, 2003). The solubility of triclosan in different solvents is summarised in Table 11 (SCCS, 2010). The partition coefficient of triclosan ($\log P_{ow} = 4.76$) suggests that it is lipophilic. The partition coefficient is a ratio of solubility between two liquids – typically octanol and water. Values of its pK_a have been reported in the range of 7.9–8.1 (Son et al., 2009). It is an antimicrobial and preservative agent applied in PCPs (toothpaste, detergent, soap, shampoos, skin care creams and lotions) with a typical concentration in the range of 0.1–0.3%. The content of triclosan in household products should not exceed 0.3% (w/w) (Allmyr et al., 2006).

However, its ubiquitous use has resulted in the release of triclosan into waste water, sediments and many water sources (Zhao et al., 2013) and its presence in waste waters may affect ecosystems and human health. In particular, triclosan can kill off algae (Tatarazako et al., 2004). There have also been several other health concerns attributed to triclosan as it can accumulate in the human body over time and may result in long-term health risks (Allmyr et al., 2006; Calafat et al., 2008). It can also degrade in the aquatic environment to make more toxic products. The most concerning degradation products are dioxins, which are known carcinogens that cause DNA mutations as well as birth defects in offspring (Geyer et al., 2002). Therefore, it is critical to develop sensitive methods for the detection and quantification of triclosan.

Table 11: Solubility of triclosan in selected solvents and chemicals (SCCS, 2010)

Solvent	Solubility at 25°C (g Triclosan/100 g solvent)
Distilled water (20°C)	0.001
Distilled water (50°C)	0.004
1 N caustic soda	31.7
1 N sodium carbonate	0.40
1 N ammonium hydroxide	0.30
Triethanolamine	> 100
Acetone	> 100
Ethanol 70% or 95%	> 100
Isopropanol	> 100
Propylene glycol	> 100
Polyethylene glycol	> 100
Methyl cellosolve (Union Carbide Corp.)	> 100
Ethyl cellosolve (Union Carbide Corp.)	> 100
Dipropylene glycol	~40
Glycerine	0.15
n-Hexane	8.5
Petroleum jelly (white, USP)	~0.5
Tween 20 (ICI America Inc.)	> 100
Tween 80 (ICI America Inc.)	> 100
Triton X-100 (Rohm & Haas)	> 100
Olive oil	~60
Castor oil	~90

4.5.1 Environmental concentrations of triclosan

Several recent studies have clearly demonstrated the widespread presence of triclosan in the environment, especially in waste water, WWTP effluents, river water and in sediments in various countries. Environmental concentrations of triclosan reported in the published literature are provided in Table 12.

Table 12: Environmental concentrations of triclosan (SCCS, 2010)

Environmental matrix	Triclosan concentration	Country	Reference
River water	Average 140 $ng \cdot L^{-1}$ Max 2300 $ng \cdot L^{-1}$	USA	(Kolpin et al., 2002)
Seawater	1.4–74.0 $ng \cdot L^{-1}$ Average 15 $ng \cdot L^{-1}$ Median 10 $ng \cdot L^{-1}$	Switzerland	(Lindström et al., 2002)
River water	600–40 000 $ng \cdot L^{-1}$	USA	(Lopez-Avila & Hites, 1980)

Environmental matrix	**Triclosan concentration**	**Country**	**Reference**
Lake sediment	< D.L – 100 000 µg kg^{-1} d.w	Switzerland	(Singer et al., 2002)
Waste water effluents	42–213 ng·L^{-1}		
Receiving water	11–98 ng·L^{-1}		
Activated sludge	580 µg kg^{-1} d.w		
Sewage influent	600–1300 ng·L^{-1}	Switzerland	(Lindström et al., 2002)
Sewage effluent	100–650 ng·L^{-1}		
Streams	< D.L–140 ng·L^{-1}	USA	(Kolpin et al., 2004)
Sewage influent	380 ng·L^{-1}	Sweden	(Bendz et al., 2005)
Sewage effluent	160 ng·L^{-1}		
Streams with known input of raw waste water	Median 120 ng·L^{-1} Max 1600 ng·L^{-1}	USA	(Glassmeyer et al., 2005)
River water	8.8–26.3 ng·L^{-1}	USA	(Zhang et al., 2007)
Waste water influent	6100 ng·L^{-1}	USA	(Halden & Paull, 2005)
Waste water effluent	35 ng·L^{-1}		
River and coastal water	4.1–117 ng·L^{-1}	China	(Chau et al., 2008)
WWTP outfall	120 ng·L^{-1}	USA	(Coogan et al., 2007)
WWTP outfall	112 ng·L^{-1}	USA	(Coogan & Point, 2008)
Waste water effluent (activated sludge)	240–410 ng·L^{-1}	USA	(McAvoy et al., 2002)
Waste water effluent (trickling filters)	1600–2700 ng·L^{-1}		
Digested sludge	530–15 600 µg·kg^{-1} d.w		
Waste water effluent	Median 106 ng·L^{-1} Mean 108 ng·L^{-1} Max 324 ng·L^{-1}	Canada	(Lishman et al., 2006)
Waste water influent	Median 1860 ng·L^{-1} Mean 1930 ng·L^{-1} Max 4010 ng·L^{-1}		
Waste water effluent	30–250 ng·L^{-1}	USA	(Waltman et al., 2006)
Waste water influent	2700-26 800 ng·L^{-1}		
Waste water influent	70 ng·L^{-1}	USA	(Heidler & Halden, 2007)
Waste water influent	4700 ng·L^{-1}		
Digested sludge	30 000 µg kg^{-1} d.w		
Waste water effluent	83–1283 ng·L^{-1}	Spain	(Kantiani et al., 2008)

Environmental matrix	Triclosan concentration	Country	Reference
Waste water influent	231–12 500 ng·L^{-1}		
Waste water effluent	190 ng·L^{-1}	Canada	(Fair et al., 2009)
Waste water influent	2830–3440 ng·L^{-1}		
Waste water effluent	180–5 370 ng·L^{-1}	USA	(Kumar et al., 2010)
Waste water influent	5 213–32 639 ng·L^{-1}		
Waste water effluent	43–59 ng·L^{-1}	Germany	(Bester, 2003)
Waste water influent	1100–1300 ng·L^{-1}		
Sewage sludge	1000–1300 µg kg^{-1} d.w		
Waste water effluent	Max 3100 ng·L^{-1} Average 69.2 ng·L^{-1} LOD 17.4 ng·L^{-1}	UK	(Kanda et al., 2003)
Waste water effluent	340–1100 ng·L^{-1}	UK	(Sabaliunas et al., 2003)
Waste water influent	7500–21 900 ng·L^{-1}		
Waste water effluent	<700 ng·L^{-1}	UK	(Thompson et al., 2005)
Waste water effluent	23–434 ng·L^{-1} Median 108 ng·L^{-1}	Australia	(Ying & Kookana, 2007)
Biosolid from WWTP	90-16790 µg kg^{-1} d.w Median 2320 µg kg^{-1} d.w		
Seawater	0.0008–6.87 ng·L^{-1}	Germany	(Xie et al., 2008)
Sea water	50 ng·L^{-1}	Japan	(Okumura & Nishikawa, 1996)
Sediment	10 µg·kg^{-1} d.w		
Marine	10 µg·kg^{-1} d.w		
River sediment	4.4 and 35.7 µg·kg^{-1} d.w	Spain	(Morales et al., 2005)
Biosolid from WWTP	5400 µg·kg^{-1} d.w		
River sediment	Max 80 µg·kg^{-1} d.w Average 70 µg·kg^{-1} d.w	USA	(Miller et al., 2008)
Biosolid from WWTP	3300–5970 µg·kg^{-1} d.w		(Chu & Metcalfe, 2007)

Environmental matrix	Triclosan concentration	Country	Reference
Biosolid from WWTP	1170–32 900 µg·kg^{-1} d.w Median 10 200 µg·kg^{-1} d.w	USA	(Kinney et al., 2006)
Biosolid from WWTP	90–7060 µg·kg^{-1} d.w	USA	(Cha & Cupples, 2009)
Soil	0.16–1.02 µg·kg^{-1} d.w		
Pore water	201–273 300 ng·L^{-1} (calculated)		(Chalew & Halden, 2009)

d.w. = dry weight

Triclosan is a frequent contaminant of aquatic and terrestrial environments and it is detected at a concentration ranging from parts-per-trillion in surface water to parts-per-million in biosolids. The elevated concentration of triclosan in biosolids and aquatic sediments may be attributed to the high usage, strong sorption to organic matter and environmental persistence of this compound (Chalew & Halden, 2009). Regarding the half-life, it depends on the environmental compartment and prevailing conditions (Bester, 2005; Halden & Paull, 2005). Fish, algae and crustaceans may be affected by elevated levels of biocides in surface waters, the concentrations of which are influenced by raw and treated sewage. It is important to note that triclosan has been detected in surface waters at levels higher than the no-observed effects concentration (NOECO) of algae. Microorganisms are generally not as sensitive to triclosan as non-target species, and the minimum inhibitory concentration threshold values for microbes is increased by environmental level of triclosan in activated sludge and biosolids, which is important for the health and fertility of agricultural soils amended with biosolids (Chalew & Halden, 2009).

4.5.2 Stability of triclosan in the environment

The US EPA (SCCS, 2010; US EPA, 2009) states that based on data from a preliminary test at 50°C, triclosan is hydrolytically stable under abiotic and buffered conditions over the pH range of 4–9. It degrades with calculated non-linear half-lives of 1.3–1.4 days in water, 53.7–60.3 days in sediment, and 39.8–55.9 days in aerobic water-sediment systems maintained in darkness at 20°C ± 2°C. The degradation is accelerated under continuous irradiation from artificial light at 25°C in aqueous solution at pH 7, with a calculated aqueous photolytic half-life of 41 minutes. Triclosan degrades swiftly in aerobic soils maintained in darkness at 20°C ± 2°C, with calculated half-lives of 2.9–3.8 days.

Triclosan has a pK_a of 7.9, which means that it exists partially in the dissociated form in the environment, which is of relevance as anions do not generally adsorb as strongly to organic carbon and clay than their neutral counterparts. It is expected that triclosan would be immobile based on an estimated K_{oc} of 9 200 in the soil. It has an estimated Henry's Law Constant of 1.5×10^{-7} $\text{atm}^{-}\cdot\text{m}^{3}\cdot\text{mole}^{-1}$, thus it is not anticipated to volatilize from soil (moist or dry) or water surfaces.

In the laboratory, triclosan degraded via aerobic soil metabolism and aerobic aquatic metabolism with half-lives of < 4 days in soils and half-lives of < 1.5 days (water layer) and up to 60 days (sediment and total system) in water-sediment systems. In aquatic environments, triclosan is envisaged to adsorb to suspended solids and sediments and may bioaccumulate (K_{ow} 4.76), posing a threat to aquatic organisms.

Hydrolysis of triclosan is not an important environmental fate process due to its stability in the presence of strong acids and bases. However, triclosan is vulnerable to degradation via aqueous photolysis, with a half-life of < 1 hour under abiotic conditions, and up to 10 days in lake water. Considering the reaction of triclosan with photochemically produced hydroxyl radicals, an atmospheric half-life of 8 hours has also been estimated (SCCS, 2010).

4.5.3 Removal of triclosan via waste water treatment

Triclosan present in various environmental media is susceptible to degradation by oxidation by ozone and chlorine in the presence of sunlight, and to biodegradation by microorganisms (SCCS, 2010). The ubiquity of triclosan usage results in the discharge of this compound to waste water, therefore the incomplete removal of triclosan from waste water in WWTPs, as well as the spread of biosolids (a by-product of waste water treatment) onto soils, lead to the widespread distribution of triclosan in soils and surface waters. Waste water treatment achieves average removal efficiencies in the range of 58–99% (NICNAS, 2009) depending on the technical capabilities of the sewage treatment systems (Table 13) (Bester, 2003; Federle et al., 2002; Kanda et al., 2003; Lindström et al., 2002; Lishman et al., 2006; Lopez-Avila & Hites, 1980; McAvoy et al., 2002; Singer et al., 2002; Ternes et al., 2004; Thompson et al., 2005). However, mass balance studies have demonstrated that triclosan exhibits significant persistence, partitioning and sequestration in biosolids. Approximately 50% ± 19% of the incoming mass of triclosan persists and becomes sequestered in biosolids produced by activated sludge treatment in conjunction with aerobic biosolid digestion in conventional WWTPs. Thus, important pathways of biocide release into the environment are WWTP effluent discharge into surface waters and the application of biosolids to land. Effluent from WWTPs contains a complex mixture of anthropogenic and natural compounds (Heidler & Halden, 2007).

Table 13: Reported triclosan removal rate (NICNAS, 2009)

Level of Treatment	Removal Rate (%)
Untreated waste water	–
Primary treatment	2–96
Secondary treatment	
Trickling Filter	58–96
Activated Sludge	55–99
Tertiary treatment	87 to ≥ 99

The US EPA has reported the occurrence of active pharmaceutical ingredients (APIs) in finished drinking water (Daughton, 2010) and triclosan was also included as an APIs and had one of the highest reported concentrations (1.2–734 ng·L^{-1}) depending on the efficiencies of the treatment processes applied (Benotti et al., 2009; Loraine & Pettigrove, 2006; Servos et al., 2007; Snyder, 2007a; Snyder, 2008; Vanderford & Snyder, 2006; Yu et al., 2007).

An intensive Australian study by Ying and Kookana (2007) discovered triclosan in 19 effluent samples. The range of triclosan in the filtered final effluents ranged from 23 ng·L^{-1} to 434 ng·L^{-1} with the mean and median concentration of 142 ng·L^{-1} and 108 ng·L^{-1}, respectively, where the WWTPs used mainly biological treatment with an activated sludge process.

Agüera et al. (2003) found triclosan concentrations varied from 400 ng·L^{-1} in April and 800 ng·L^{-1} in May to 22 100 ng·L^{-1} in June and 19 600 ng·L^{-1} in July in Spain from a biological WWTP in effluent samples. This wide variation from month to month may be attributed to the difference in input load.

Triclosan concentrations in the effluents of seven Switzerland WWTPs ranged from 42 ng·L^{-1} to 213 ng·L^{-1} with an average concentration of 116 ng·L^{-1} (Singer et al., 2002).

The reported triclosan concentrations in WWTP effluents from the USA and United Kingdom varied from 35 ng·L^{-1} to 2700 ng·L^{-1} with an average of 1180 ng·L^{-1} in USA (n = 5) (Halden & Paull, 2005; McAvoy et al., 2002), and from 340 ng·L^{-1} to 1100 ng·L^{-1} with an average of 753 ng·L^{-1} in UK (n = 4) (Sabaliunas et al., 2003) (Figure 27).

McAvoy et al. (2002) found that triclosan concentrations in the final effluents from two WWTPs in the USA using activated sludge technology ranged between 240 ng·L^{-1} and 410 ng·L^{-1}, which was much lower than in the effluents from the other two WWTPs using trickling filter technology (which ranged from 1600 ng·L^{-1} to 2700 ng·L^{-1}). The triclosan concentrations in the influents were in the same range for the four WWTPs examined by McAvoy et al. (2002). Activated sludge systems normally have longer hydraulic times (15 h) than trickling filters (1 h to 4 h) (Thompson et al., 2005), thus achieving higher removal efficiency for triclosan in activated sludge. Overall, waste water treatment technology could be a crucial factor for triclosan levels in final effluent, and therefore in water systems.

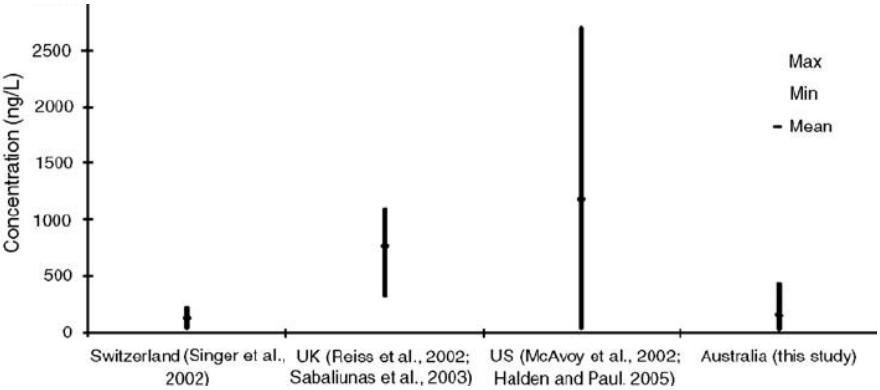


Figure 27: Comparison of triclosan levels in WWTPs effluents from Switzerland, UK, US and Australia (Ying & Kookana, 2007)

4.5.3.1 Degradation of triclosan during water chlorination and ozone treatment

Cleavage of the ether bond and chlorination of the phenolic bond were identified as the main degradation pathways for triclosan. Therefore, to determine these pathways, triclosan has been added to chlorine spiked ultrapure water or to chlorinated tap water, which led to the formation of two tetra- and one penta-chlorinated hydroxylated diphenyl ether, as well as 2,4-dichlorophenol. Chlorination of the phenolic ring and cleavage of the ether bond may also occur (Canosa et al., 2005). Free chlorine mediated oxidation of triclosan results in the formation of chloroform and other chlorinated organics (Fiss et al., 2007; Rule et al., 2005). Degradation pathways of triclosan are shown in Figure 28.

Both 2,4-dichlorophenol (2,4-DCP) and 2,4,6-trichlorophenol (2,4,6-TCP) are more stable than triclosan, which means that although triclosan can naturally degrade into these by-products, it cannot reform from these products spontaneously. 2,4,6-TCP is a known endocrine disrupter, and may cause cancer, birth defects and developmental disorders in offspring. 2,4-DCP may be fatal if large amounts are absorbed by the body (Geyer et al., 2002).

Treatment with ozone during municipal sewage treatment was efficient at the removal of triclosan (Dodd et al., 2009; Suarez et al., 2007; Wert et al., 2009). Although chloramines can be employed, chlorine is a stronger oxidant and has been shown to be more effective at oxidising pharmaceuticals and EDCs (Snyder et al., 2007b).

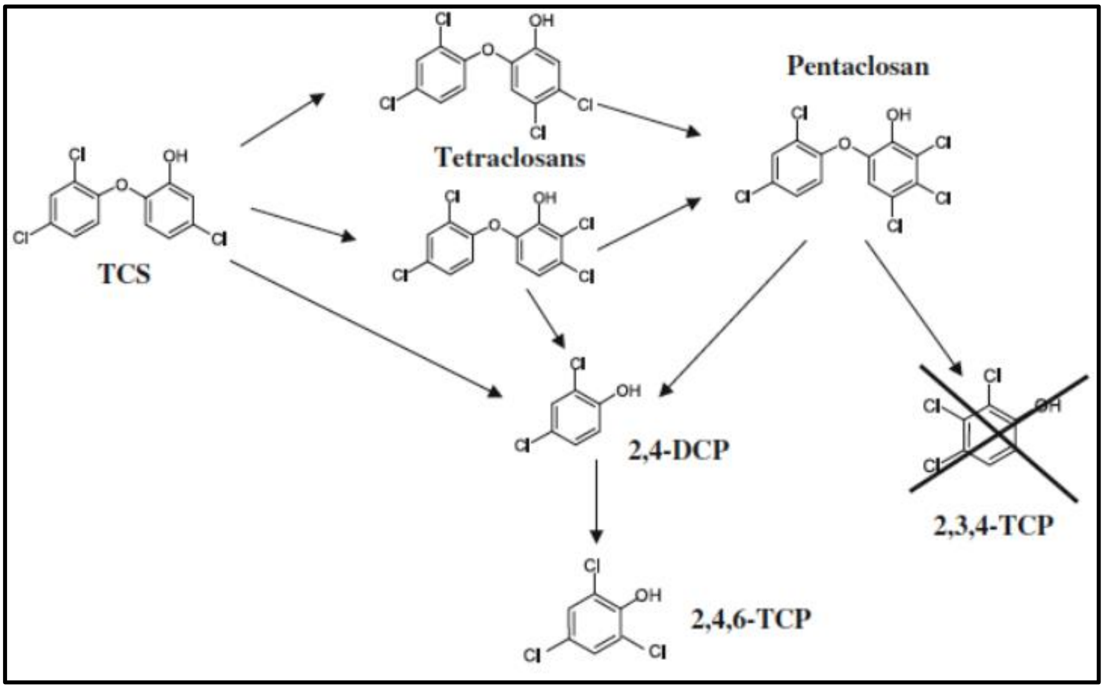


Figure 28: Degradation pathway of triclosan in the presence of free chlorine (2,3,4-TCP was not detected as a degradation product) (Aufiero, 2012)

A comprehensive survey was done by Benotti et al. (2009) for 20 pharmaceuticals, 25 known or potential EDCs, and six other waste water contaminants in source water (n = 19), finished drinking water (n = 18), and distribution system (tap) water (n = 15) from 19 US drinking water treatment plants sampled during 2006–2007. Median concentrations of triclosan were less than 10 ng·L^{-1} (3.0, 1.2 and < method reporting limit) for source, finished and distribution water respectively). The triclosan was extracted by solid phase extraction (SPE) and analysed by LC–MS/MS and gas chromatography–tandem mass spectrometry (GC–MS/MS).

Triclosan was detected in six source water samples and one finished water sample and was not in distribution waters that had been efficiently oxidised by either chlorine or ozone. Moreover, triclosan finished water concentrations were lower than source water concentrations. Triclosan is removed by either chlorine (80–96%) (Gibs et al., 2007; Snyder et al., 2007b; Westerhoff et al., 2005) or ozone oxidation (92–95%) (Snyder et al., 2007b; Westerhoff et al., 2005).

4.6 Application of Nanoparticles to Determine Acetaminophen (Paracetamol) in Water

Paracetamol or acetaminophen (N-acetyl-p-aminophenol) with a molecular weight of 151.17 g·mol^{-1} and a log K_{ow} of 0.46 (Westerhoff et al., 2005) is an acylated aromatic amide that was first introduced by Von Mering in 1893 as an antipyretic/analgesic medicine. It has a pK_a of 9.38 with a solubility of 1.4 × 10^4 mg·L^{-1} in water at 25°C (Achilleos et al., 2008). The structure of acetaminophen is shown in Figure 29.

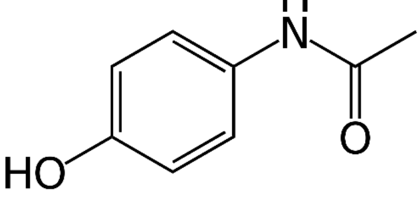


Figure 29: Structure of acetaminophen

Acetaminophen unlike other common analgesics, such as aspirin and ibuprofen, has relatively little anti-inflammatory activity. As a result, it is not considered to be an NSAID. Acute overdoses of paracetamol can cause potentially fatal liver damage, and in some individuals, a normal dose can cause the same and the risk can be heightened by alcohol consumption. Recent studies have suggested that excessive doses and/or excessively high plasma concentrations of acetaminophen may be associated with hepatotoxicity (Ameer & Greenblatt, 1977; Barker et al., 1977).

4.6.1 Environmental concentration of acetaminophen

Several investigations are being done to determine the concentrations of different pharmaceutical compounds found in surface water to be able to predict potential negative effects on the living organisms. Acetaminophen is usually easily biodegraded in biological WWTPs (Ternes, 2006), although maximum concentrations of acetaminophen in surface waters of 10 000 $ng\cdot L^{-1}$ have been found (Daughton & Ternes, 1999).

A comprehensive method was developed for 16 pharmaceutical compounds using SPE of water samples, followed by LC–MS/MS in the USA (Wang et al., 2011). The results showed that acetaminophen had one of the highest pharmaceutical concentrations in untreated water at a concentration of 70 $ng\cdot L^{-1}$.

4.6.2 Removal of acetaminophen by waste water treatment

The primary sources of pharmaceuticals entering surface water are from treated or untreated municipal waste water effluent discharges into receiving surface water bodies (Buser et al., 1998; Buser et al., 1999; Daughton, 2001; Heberer et al., 2001; Heberer et al., 2002; Kolpin et al., 2002; Ternes, 1998), improper disposal of pharmaceutical waste and excess medication by consumers, and health care and veterinary facilities into sewers and drains.

Roberts and Thomas (2006) analysed waste water effluent and surface waters for 13 pharmaceuticals by reversed-phase high-performance liquid chromatography–electrospray tandem mass spectrometry following SPE. Acetaminophen was detected at concentrations ranging from 11 $ng\cdot L^{-1}$ to 69 570 $ng\cdot L^{-1}$ in raw effluent. The treatment process was found to remove 100% of the paracetamol present in the raw effluent.

Daughton (2010) also detected acetaminophen in over 5% of groundwater samples, where it was one of the APIs with the highest reported concentrations ranging from 0.3 $ng\cdot L^{-1}$ to 210 $ng\cdot L^{-1}$ (Naidenko et al., 2008; Stackelberg et al., 2007; Togola & Budzinski, 2008; Versteegh et al., 2007).

Little data has been published on the removal of APIs by full-scale reverse osmosis. Radjenović et al. (2008) studied APIs handled by drinking water treatment plants using reverse osmosis and nanofiltration. Of the 31 APIs targeted in the source groundwater, 12 APIs (acetaminophen, carbamazepine, diclofenac, gemfibrozil, glibenclamide, hydrochlorothiazide, ketoprofen, mefenamic acid, metoprolol, propyphenazone, sotalol, sulfamethoxazole) were frequently detected at average concentrations ranging from 4.3 $ng\cdot L^{-1}$ (sotalol) to 137 $ng\cdot L^{-1}$ (ketoprofen), with maximum concentrations of up to hundreds of $ng\cdot L^{-1}$ (carbamazepine, diclofenac, gemfibrozil, ketoprofen, propyphenazone) or thousands of $ng\cdot L^{-1}$ (hydrochlorothiazide).

4.7 Monitoring Methods for Triclosan

The number of reported fluorescence-based monitoring methods for triclosan is limited, therefore additional sensor based techniques that use other means of detection are also discussed in this section.

4.7.1 Electrochemical methods

4.7.1.1 Mercury electrode

Safavi et al. (2003) determined the trace amount of triclosan in toothpaste (containing 0.3% triclosan), waste water liquid soap and samples containing Triton X-100 by differential pulse voltammetry. Under optimum conditions of pH 7, an accumulation potential of −450 mV and accumulation time of 90 s, the determination was based on the adsorption of triclosan on a hanging drop mercury electrode. Over the range 2.5–60 $\mu g \cdot L^{-1}$, the peak current was proportional to the concentration of triclosan with a detection limit of 1.9 $\mu g \cdot L^{-1}$ and relative standard deviation (RSD) lower than 3%. The procedure was validated for real samples and recoveries were 78–110%.

4.7.1.2 β-CD/GNP coated electrode

Li et al. (2014) applied β-CD and graphene nanoplatelets (GNPs) to construct a β-CD/GNP-coated electrode for the determination of triclosan. The large surface area of GNPs, fast electron transfer processes on GNPs, as well as the unique spacious structure of β-CD, allowed for the sensitive detection of triclosan. Monitoring of triclosan in the range of 2.0 µM to 120 µM was achieved by differential pulse voltammetry with a detection limit of 0.6 µM. The recovery of triclosan in water samples was in the range of 98.4–99.1% with RSD values of 1.3–3.0%.

4.7.1.3 Gold nanoparticle/polyoxometalate/reduced graphene oxide

A novel molecular imprinted electrochemical sensor based on AuNPs decorating polyoxometalate (POM; $H_3PW_{12}O_{40}$)/reduced graphene oxide (rGO) to determine trace triclosan in waste water was designed by Yola et al. (2015).

The formation process was based on functionalising rGO with POM (as reducing and stabilising reagent) through electrostatic interactions to produce a photocatalyst (POM/rGO) in aqueous solution. AuNPs were further deposited on the POM/rGO without using any reducing agent and the prepared nanomaterial (AuNPs/POM/rGO) was employed to modify a glassy carbon electrode (GCE) (AuNPs/POM/rGO/GC) under infrared light.

In the next step, a triclosan MIP film was prepared on the AuNPs/POM/rGO/GCE (MIP/AuNPs/POM/rGO/GC), which was prepared by cyclic voltammetry (CV) using phenol as a monomer in a phosphate buffer solution (pH 7.0) containing triclosan. To break up the electrostatic interactions between the phenol monomer and polar groups of the triclosan, NaCl was used as desorption agent in a batch system. Limit of quantification (LOQ) and LOD of triclosan were found to be 5.0×10^{-10} M and 1.5×10^{-10} M, respectively. The differential pulse voltammograms at different concentrations of triclosan showed that the current peak increased with increasing concentration of triclosan, with a linear detection range of 0.5–50.0 nM. The RSD was 0.3% in 10.0 nM triclosan. Recoveries in waste water and lake water samples were 98.9–100%.

Table 14 summarises and compares the linear ranges and LODs for the detection of triclosan achieved on the carbon nanomaterials.

Table 14: Comparison of linear ranges and LODs obtained using nanomaterial-based electrode systems for triclosan

Nanomaterials	Detection method	Linear range (μM)	LOD (μM)	Reference
CNP-PDDAC	CV	0.5–50	–	(Amiri et al., 2007)
Carbon nanoparticles	CV	1.0–120	10	(Vidal et al., 2008)
MWCNTs	CV	0.17–6.0	0.057	(Yang et al., 2009)
Carbon nanodot	CV and EIS	0.01–1000	9.2×10^{-3}	(Dai et al., 2012)
β-CD/GNP	CV	2.0–120	0.6	(Li et al., 2014)
MIP/AuNPs/POM/rGO/GC	CV	0.0005–0.05	1.5×10^{-4}	(Yola et al., 2015)

CNP-PDDAC – Carbon nanoparticles-poly(diallyldimethylammonium chloride)
CV – Cyclic voltammetry
β-CD/GNP – β-Cyclodextrin graphene nano platelets
MWCNTs – Multiwall carbon nanotube
MIP – Molecular imprinted polymer
AuNPs – Gold nanoparticles
POM – Polyoxometalate
rGO – Reduced graphene oxide
GC – Glassy carbon electrode
EIS – Electrochemical impedance spectroscopy

4.7.2 Molecular imprinted polymers

4.7.2.1 Core-shell MIPs on silica-coated MWCNTs

Gao et al. (2010) synthesised core-shell MIPs on silica-coated multiwall carbon nanotubes (MWCNTs) via a sol-gel process for the determination of triclosan in environmental water samples. They used a molecular imprinting technique to prepare MIPs as selective sorbents (Ge & Turner, 2009).

The monomers 3-aminopropyltriethoxysilane (APTES), phenyltrimethoxysilane (PTMOS), and a 1:1 (v/v) mixture of APTES and PTMOS were selected as functional monomers to examine the capacity of the corresponding polymers. They found that APTES alone was far better than PTMOS as well as the mixture of APTES and PTMOS, not only in adsorption capacity, but also in imprinting efficiency due to the interaction between functional monomers and the template molecule triclosan. The monomer APTES provides amine groups that can form hydrogen bonds with O atoms and interact with hydroxyl groups through acid/base interaction, and also provides the hydrophobic ether-like Si–O–Si bond (Fireman-Shoresh et al., 2003); while PTMOS supplies the phenyl group for π-π interactions with the phenyl residue.

In this regard, triclosan has two O atoms, which can form multiple hydrogen bonds with the $-NH_2$ of APTES and the acid/base interaction. The hydrogen bonds and the acid/base interaction between functional monomers of APTES and triclosan are far stronger than π-π interactions between triclosan and PTMOS. As a result, APTES was chosen as the functional monomer.

The binding isotherms of triclosan to CNTs@triclosan-MIPs were determined in the concentration range of 0.01–40 $\mu g \cdot mL^{-1}$. The recoveries of triclosan in river water and lake water samples ranged from 92.1% to 95.3% and 90.7 to 93.6%, respectively. The synthetic route for CNTs@triclosan-MIPs is shown in Figure 30.

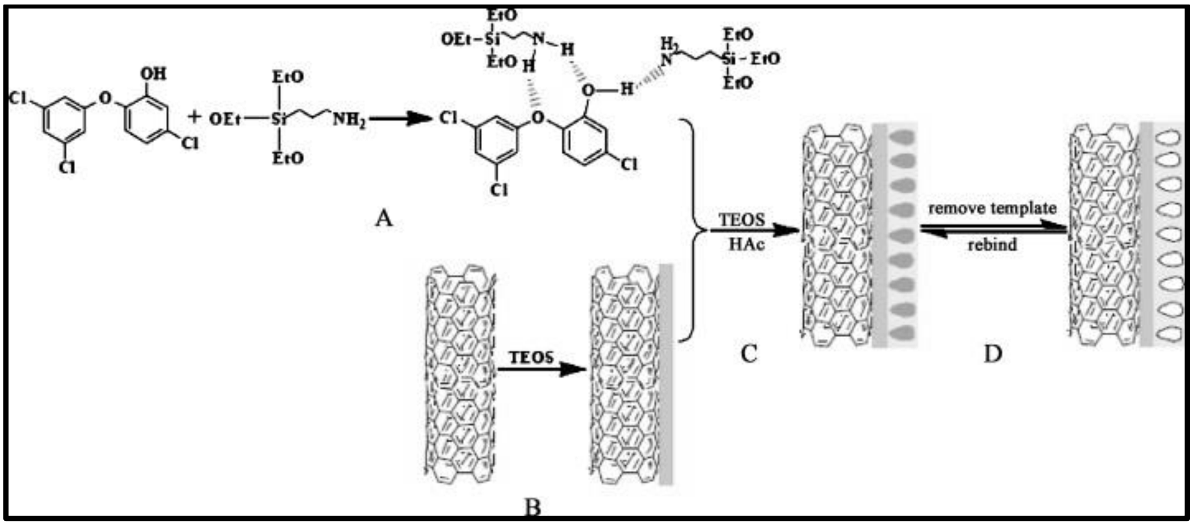


Figure 30: Scheme of the synthetic route for CNTs@triclosan-MIPs: (a) Formation of template (triclosan)-amino silica monomer (APTES) complex; (b) Transformation of the surface of purified CNTs to silica shell by a sol-gel process using TEOS and APTES in the presence of CTAB to obtain core@shell CNTs@SiO$_2$; (c) Reaction of CNTs@SiO$_2$ with template silica monomer complex to produce silica surface functionalised with triclosan-imprinted polymer; (d) Removal of the triclosan from polymer shells to obtain the CNTs@triclosan-MIPs (Gao et al., 2010)

4.7.2.2 Triclosan-imprinted poly(2-hydroxyethyl methacrylate-methacryloylamidoglutamic acid surface plasmon resonance nanosensor

Surface plasmon resonance (SPR) is an optical phenomenon, which occurs when p-polarized light goes through a prism and hits a metal layer covering the prism surface at a particular angle (Yola et al., 2014).

Triclosan-imprinted poly(2-hydroxyethyl methacrylate-methacryloylamidoglutamic acid [p(HEMAGA)] SPR nanosensor was developed as a novel molecular imprinted SPR chemical sensor for the sensitive and selective detection of triclosan in waste water (Atar et al., 2015), using an allyl mercaptan modified gold SPR chip and p(HEMAGA) nanofilm. They used methacryloylamidoglutamic acid (MAGA) as a monomer and triclosan as the template.

Electrostatic interactions and hydrogen bonding occurs between the carboxylic acid groups of the MAGA monomer and polar groups of triclosan molecules. To break the interactions, they used 1.0 M NaCl aqueous solution as desorption agent. The optimum response signal was observed at pH 6.0, which indicates that the polymerization of the SPR surface increased to this value. The MAGA monomer's carboxylic acid groups were charged negatively and interacted with the OH groups in the structure of triclosan with increasing pH values. Triclosan is mostly stable in acidic medium but increasing the pH can reduce the stability of triclosan. The ionization was more noticeable at higher pHs, as the anion concentration of triclosan rises when the pH exceeds 6.0. Therefore, triclosan binding to the SPR surface could be difficult.

The linearity range and LOD of triclosan were found to be 0.05–1.0 ng·mL^{-1} (ΔR (SPR signal) versus concentration of triclosan) and 0.017 ng·mL^{-1}, respectively with the LOQ = 0.05 ng·mL^{-1}. The triclosan-imprinted SPR chip (MIP) was 15.2 and 15.9 times more selective for triclosan than triclocarban and methyl triclosan, respectively, which are potential interferents. The recovery rates were 98–102% for the triclosan-imprinted SPR nanosensor for triclosan in waste water.

4.7.3 Chromatography/mass spectrometry

A number of chromatography methods coupled with mass spectroscopy have been proposed to detect triclosan, but they usually have significant disadvantages in terms of analytical running costs, complexity, environmentally unfriendly solvent use, expensive instrumentation, long analysis time as well as the requirements for highly skilled technicians and laborious sample pretreatment (Gupta et al., 2015a; Gupta et al., 2015b; Mergu & Gupta, 2015). Rapid and sensitive screening methods may therefore provide desirable alternatives. Table 15 compares the linear ranges and LODs for the detection of triclosan achieved by chromatography-based methods to compare the screening methods discussed.

Table 15: Comparison of the sensitivity of chromatography-based methods for triclosan determinations in water samples

Method	Extraction method	Linear range (µM)	LOD (µM)	LOQ (µM)	Samples	Ref.
GC–MS	Liquid–liquid extraction	1.7×10^{-4}–5.2×10^{-4}	1.0×10^{-4}–2.0×10^{-4}	–	River and seawater	(Okumura & Nishikawa, 1996)
LC–MS/MS	SPE Oasis HLB	7.0×10^{-4}–7.0×10^{-1}	7.0×10^{-4}	2.3×10^{-3}	Surface and waste waters	(Quintana & Reemtsma, 2004)
GC–MS/MS	C18 SPE cartridge	5.0×10^{-4}–7.3×10^{-4}	9.0×10^{-7}	–	Waste, river, seawater	(Wu et al., 2007)
GC–MS/MS	Oasis HLB sorbent	3.4×10^{-5}–3.4×10^{-3}	7.0×10^{-6}	3.0×10^{-5}	Hospital effluent	(Gómez et al., 2007)
GC–MS	SPE (GF/F glass fibre filter)	0.34–34.5	4.5×10^{-4}	1.5×10^{-3}	Waste water	(Gatidou et al., 2007)
GC–MS	Oasis HLB cartridge	6.2×10^{-5}–8.0×10^{-2}	3.5×10^{-7} 3.5×10^{-5}	–	Spring water Waste water	(Gibson et al., 2007)
GC–MS	Oasis HLB cartridge	1.7×10^{-5}–7.0×10^{-4}	$<2.0 \times 10^{-5}$ $<3.5 \times 10^{-5}$	–	Drinking water Surface water	(Yu et al., 2007)
LC–DAD	Stir bar sorptive extraction and liquid desorption (SBSE–LD)	1.4×10^{-3}–3.7×10^{-1}	3.4×10^{-4}	–	Urban waste water	(Silva & Nogueira, 2008)
GC–MS/MS	SPE (C18 cartridge)	1.4×10^{-5}–0.4×10^{-3}	7.0×10^{-6}	–	River and coastal water	(Chau et al., 2008)

GC–MS	Gas chromatography–mass spectrometry
GC–MS/MS	Gas chromatography–tandem mass spectrometry
LC–DAD	Liquid chromatography–diode array detection
SPE	Solid phase extraction

4.7.4 Spectrophotometric methods

4.7.4.1 Azo compound formation

Lu et al. (2009) developed a simple method to determine triclosan in PCPs including toothpastes, disinfection solution and handwash. The determination was based on the reaction of sodium nitrite with *p*-sulfanilic acid in an acidic medium to form diazonium ion, with which triclosan further formed an azo compound in alkaline medium (Figure 31).

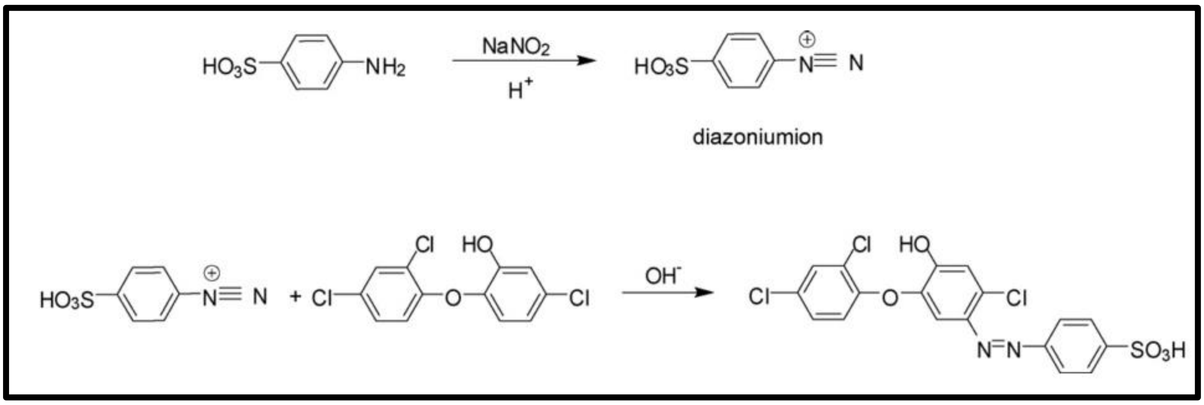


Figure 31: Reaction mechanism of chromogenic species with triclosan by means of a diazotization reaction (Lu et al., 2009)

This reaction took place at room temperature and the final yellow colour of the derivate remained stable for at least 1 h. Absorbance versus the concentration of triclosan was linear over the range of 0–30 $\text{mg}\cdot\text{L}^{-1}$ with the LOD and molar absorptivity (ε) of 0.079 $\text{g}\cdot\text{L}^{-1}$ and 8.27×10^3 $\text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$ respectively. The practical feasibility of the method was tested for several PCPs and the recoveries of spiked samples were between 92.1% and 100.0%. The accuracy of the method was satisfactory when evaluated by HPLC for real samples.

4.7.4.2 Chemiluminescence methods

A highly sensitive and selective method was proposed by Song et al. (2007), based on the phototransformation of triclosan to a light-emitting precursor in the presence of fluorescein in alkaline medium. The chemiluminescence reaction was then started by a strong base or oxidants such as N-bromosuccinimide, which developed during online phototransformation-flow injection. After mixing with oxidant, the light produced was detected by a photomultiplier. Under optimum conditions, the log of the chemiluminescence intensity was linear to the concentration of triclosan over the range of 8.0×10^{-8} to 1.0×10^{-4} $\text{mol}\cdot\text{L}^{-1}$ and the detection limit was 5.0×10^{-8} $\text{mol}\cdot\text{L}^{-1}$. The proposed method was validated for the determination of triclosan in toothpaste samples, which were prepared by extraction with 0.01 $\text{mol}\cdot\text{L}^{-1}$ sodium hydroxide solution and the results were compared to HPLC as a standard method.

4.8 Monitoring Methods for Acetaminophen

4.8.1 Fluorescence spectroscopy

4.8.1.1 CdS nanocrystals

CdS nanocrystals as luminescent probes to determine acetaminophen were developed by Shakeri et al. (2011). They investigated the interactions between CdS nanoparticles and acetaminophen by using molecular absorption and fluorescence spectroscopy techniques. The fluorescence intensity of CdS nanoparticles is related to the concentration of the quencher (Q), as it decreased with increasing amount of acetaminophen in solution. This was attributed to collisional or dynamical quenching and static quenching. The dynamic fluorescence quenching can be described by the Stern–Volmer equation as follows:

$$\frac{l_0}{l} = 1 + K_{sv}[Q]$$

Where K_{SV} is quenching constant or Stern–Volmer constant. Under optimum conditions, the extent of fluorescence quenching of CdS QDs is linearly proportional to the concentration of acetaminophen.

4.8.1.2 L-cys-capped CdTe nanoparticles

The use of QDs as optical probes in the analytical field has been extensively exploited after the first application of QDs in biological systems by Nie's and Alivisatos's groups independently in 1998 (Xu et al., 2011). In 2012, a simple and sensitive assay of paracetamol was proposed by Li et al. (2012a) based on the quenching of the fluorescence intensity of L-cys-capped CdTe nanoparticles in aqueous solution. It relies on changes in the fluorescence intensity when the target analyte interacts with the surface of QDs which influences the efficiency of radiative recombination, leading to either PL activation or quenching (Frasco & Chaniotakis, 2010).

They prepared a set of solutions containing 500 µL L-cys-capped CdTe nanoparticles stock solution and different amounts of freshly prepared paracetamol standard solutions, in 25 mL phosphoric buffer solution (pH 8). Fluorescence spectra were measured and recorded after 10 min.

L-cys is a multifunctional ligand, which possesses a thiol group to strongly bond Cd^{2+} on the surface of the nanoparticles by chemical bonding, and hydrophilic amine and carboxyl groups allow for hydrogen bond attachment to each other or the paracetamol molecules (Kuang et al., 2010).

A significant luminescence quenching with no optical shift of emission was observed in the L-cys-capped CdTe nanoparticles solution upon paracetamol introduction. The quenching may be due to hydrogen bond formation between L-cys and paracetamol on the surface of the nanoparticles. As a matter of fact, hydrogen bonding leads to L-cys molecules being removed from the surface of the nanoparticles, and these surface changes of CdTe nanoparticles induce the fluorescence quenching (Kuang et al., 2010). Under optimal experimental conditions, the relative fluorescence intensity ($\Delta I = I_0/I$) of L-cys-capped CdTe nanoparticles versus the concentration of paracetamol was linear over the range of 0.01–0.016 µM with a detection limit of 0.0042 µM. The applicability of the method was tested in pharmaceutical tablets. Table 16 compares the linear ranges and detection limits for the detection of paracetamol achieved by fluorescence methods.

Table 16: Comparison of the sensitivity of different fluorescence methods for paracetamol

Method	Linear Range	LOD	LOQ	Sample	Reference
Solid phase fluorescence	100–400 mg·g^{-1}	13.0–16.7 mg·g^{-1}	43.1–55.7 mg·g^{-1}	Powdered pharmaceutical	(Moreira et al., 2005)
Fluorescence study of acetaminophen binding to serum albumin bovine	30–1000 µM	–	–	–	(Bojko et al., 2009)
L-cys-capped CdTe nanoparticles	0.01–0.016 µM	0.0042 µM	–	Pharmaceutical tablets	(Li et al., 2012a)

4.8.2 Electrochemical methods

4.8.2.1 SWCNT–GNS/GCE

A single-walled carbon nanotube (SWCNT) graphene nanosheet (GNS) hybrid film modified GCE was prepared by Chen et al. (2012b) and used for the electrochemical detection of acetaminophen. Firstly, GCEs were polished by alumina powder then GNS, SWCNT and SWCNT–GNS (mass ratio of 3:1) suspensions (in dimethylformamide) were separately dropped onto the surface of clean GCEs. Cyclic voltammogram of acetaminophen were recorded of bare GC, GNS/GC, SWCNT/GC, and SWCNT–GNS/GCEs in 0.1 M phosphate-buffered saline (PBS) (pH 7.0). With the addition of acetaminophen at the modified electrodes, the oxidation and reduction peak currents of acetaminophen significantly increased with lower over-potential. They speculated that the remarkably enhanced oxidation current of acetaminophen at the hybrid electrode was due to not only a rise in the active surface area, but also the multi-modal pore structure. It is noteworthy to mention that the pore size distribution of SWCNT–GNS hybrids was very wide from 14.1 to 169 nm, which showed multi-modal architectures based on different combinations of mesopores and macropores (Walcarius & Kuhn, 2008; Wang et al., 2008).

The electrochemical sensor based on the hybrid modified electrode exhibited excellent analytical performance for acetaminophen detection in neutral solution with a low detection limit of 0.038 µM and a wide linear range of 0.05–64.5 µM. The recovery of acetaminophen in human blood serum samples ranged between 97.1% and 102.1%. The nanohybrid of SWCNTs and GNSs could dramatically reduce the aggregation and stacking between SWCNTs or GNSs, which resulted in enhanced surface area and wide porous structure of the SWCNT–GNS hybrids.

4.8.2.2 MIPs based on electropolymerization of p-phenylenediamine

Pontie et al. (2014) developed MIPs based on electropolymerization of p-phenylenediamine in a PBS at pH 7.4 for the direct quantification of acetaminophen in pharmaceutical tablets using CV and square wave voltammetry (SWV). A linear response between the current and acetaminophen concentration was found in the range from 0.0001 to 500 mg·L^{-1} with a LOD of 30 µg·L^{-1} (or 200 nM).

4.8.2.3 Self-assembly micellization of amphiphilic copolymers

Luo et al. (2013a) imprinted paracetamol templates into copolymer micelles during the self-assembly micellization of amphiphilic copolymers through the interactions between paracetamol and copolymer chains aided by hydrophobic effects. The MIP micelles were then deposited onto the electrode to form a MIP film via direct electrodeposition, and subsequent photo-cross-linking locked the recognition cavities in the polymer matrix and improved the film’s robustness. Finally, the removal of the paracetamol template from the film resulted in spatially organised cavities to generate the MIP electrode, which has affinity and selectivity towards the analyte. Thus, MIP micelles were applied as the molecular recognition element in an electrochemical MIP-based sensor for voltammetric selective and sensitive detection of paracetamol.

Through hydrogen bonds between the ester and carbamate groups in the copolymer and hydroxyl and amide groups in paracetamol, three-dimensional micelle structures around the paracetamol molecule were introduced and produced numerous binding sites. In addition, hydrophobic effects played a pivotal role in driving paracetamol into the copolymer micelle.

An incubation time of 350 s was used to determine paracetamol on the MIP electrode. The calibration curve for the peak current versus the concentration of paracetamol exhibited a linear response ranging from 1 µM to 4 mM for the MIP sensor with a detection limit of 0.33 µM. The recovery from three paracetamol tablets varied from 95.3% to 98.9%. Photo-cross-linking not only improved the solvent resistance of the MIP film, but also locked the size and shape of the recognition cavities to provide excellent stability and repeatability. The fabrication mechanism is depicted in Figure 32.

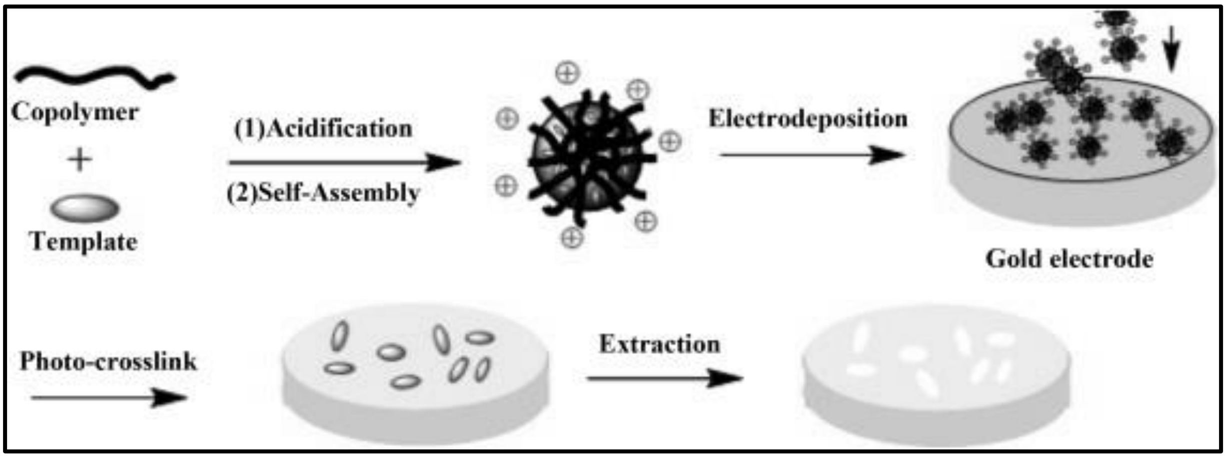


Figure 32: Schematic illustration of the MIP sensor fabrication using the self-assembly MIP micelles and electrodeposition technique (Luo et al., 2013a)

4.8.2.4 MIP micelles

Wang et al. (2013c) synthesised a novel anionic amphiphilic polymer and employed it to prepare MIP micelles via macromolecule self-assembly of an amphiphilic photo-crosslinkable copolymer using paracetamol. The resultant MIP micelles were then integrated on a GCE by anode electrodeposition to fabricate a novel electrochemical sensor for detecting paracetamol. Firstly, the amphiphilic photo-crosslinkable copolymer was self-assembled from a random coil conformation into a negatively charged micelle with paracetamol as the template molecule, which was entrapped into the resultant micelles through the interactions between paracetamol and the copolymer chain as well as hydrophobic effects. The resultant MIP micelles were then deposited on the surface of a GCE via anodic electrodeposition to form the MIP micelle film. Finally, cavities for paracetamol (the recognition sites) were formed by extracting the templates. Selectivity studies were carried out using similar structures to paracetamol including ascorbic acid and dopamine. The current response of the MIP sensor towards paracetamol was higher than for ascorbic acid and dopamine. Therefore, the MIP sensor gave good selectivity for the target analyte, due to the size, shape and functional group distribution of recognition sites which thus cannot bind to other analogues. The differential pulse voltammetry peak currents of the imprinted sensor increased with increasing paracetamol concentration and exhibited a wide linear response range from 10 to 8000 µM for the MIP sensor. The detection limit was estimated to be 1.0 µM. The recoveries were found to be in the range of 95.7–102% in urine samples.

4.8.2.5 MIP/GNPs/MWNTs/GCE

Zhu et al. (2013) combined MIPs, MWCNs and gold nanoparticles (GNPs), using a sol-gel technique to fabricate a new kind of electrochemical sensor for paracetamol determination. They used a conventional three-electrode system. Firstly, they deposited MWNTs onto the bare GCE surface and then deposited GNPs on the MWNTs/GCE surface to get GNPs/MWNTs/GCE. An organic–inorganic hybrid sol was prepared by mixing methyltrimethoxysilane (MTMOS) and PTMOS in a mixed solvent of ethanol and water, and then paracetamol was added to obtain an imprinted sol (Figure 33). A CV method was employed after fabrication of an imprinted sensor by immersing GNPs/MWNTs/GCE into the aforementioned to form an imprinted sol via an electropolymerization method. Then the embedded paracetamol molecules were extracted by KCl in PBS (pH 7.0) for several cycles until no obvious oxidation peak of paracetamol could be observed, giving MIPs modified GNPs/MWNTs/GCE (MIPs/GNPs/MWNTs/GCE).

PTMOS and MTMOS were selected as functional monomers in this work because they could form π-π interactions with the aromatic rings and introduce additional hydrophobicity and stability to the resultant materials (Zhang et al., 2010c). The linear range was from 0.08 µM to 50 µM with a correlation coefficient of 0.998. The detection limit for monitoring paracetamol was found to be 0.04 µM. A standard deviation of 1.4% was obtained for the reproducibility analysis of 30 µM paracetamol in PBS (pH 7.0) and 0.1 mol·L^{-1} KCl.

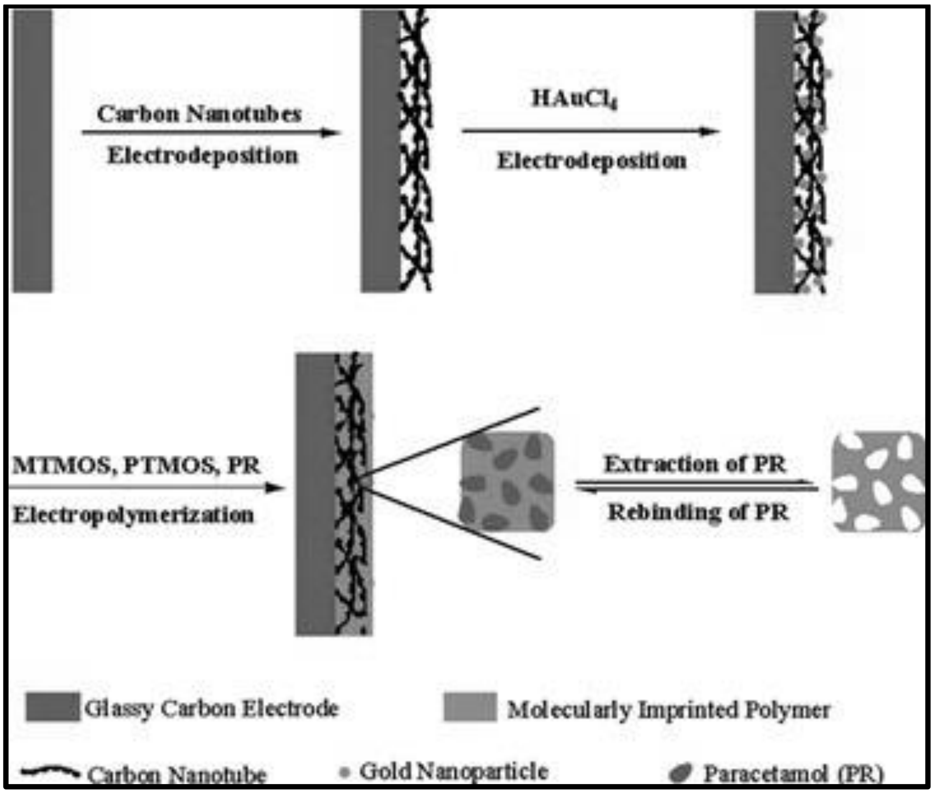


Figure 33: Schematic illustration of the fabrication procedure for MIPs/GNPs/MWNTs/GCE (Zhu et al., 2013)

Table 17 summarizes and compares the linear ranges and detection limits for acetaminophen achieved by various electrochemical sensors for comparison purposes. It is evident that most applications have been for the direct analysis of the pharmaceutical and not for water determinations.

Table 17: Comparison of the performance of electrochemical sensors for paracetamol sensing

Sensors [a]	Detection method	Linear range (µM)	Detection limit (µM)	Sample	Ref.
MIP-BAW	Dynamic quartz crystal impedance analysis	0.05–100	0.05	Human serum and urine	(Tan et al., 2001)
MIP-CFME	CV, SWV	6.5–2000	1.5	–	(Gómez-Caballero et al., 2005)
C60/GCE	CV	50–1500	5	Pharmaceutical tablets, urine	(Goyal & Singh, 2006)
PANI-MWCNTs/GCE	CV, SWV	1–100	0.25	Chlorzoxazone tablets	(Li & Jing, 2007)
C–Ni/GCE	CV, DPV	7.8–110	2.3	Pharmaceutical tablet	(Wang et al., 2007b)
MIP/PGE	DPV	5–500	0.79	Pharmaceutical tablets, syrup	(Özcan & Şahin, 2007)
Carbon film resistor electrode	CV, amperometry	0.8–500	0.14	Pharmaceutical tablets	(Felix et al., 2007)

Sensors [a]	Detection method	Linear range (μM)	Detection limit (μM)	Sample	Ref.
SWCNT–DCP/GCE	CV, SWV	0.1–20	0.04	Pharmaceutical tablets	(Sun & Zhang, 2007)
PAY/nano-TiO2/GCE	CV	12–120	2.0	Commercial tablets	(Kumar et al., 2008)
TCPE/CILE	CV, DPV	1–2000	0.3	Pharmaceutical tablets, Urine	(ShangGuan et al., 2008)
MWCNT–BPPGE	AdsSV	0.01–2 2–20	0.01	Pharmaceutical tablets	(Kachoosangi et al., 2008)
Boron-doped diamond electrode	SWV, DPV	0.5–83	0.49	Pharmaceutical tablets	(Lourencao et al., 2009)
CNTs modified screen printed electrode	DPV, SWV, ACV	2.5–1000	0.1	Pharmaceutical tablets	(Fanjul-Bolado et al., 2009)
GNMCPE	Amperometry, CV	0.66–529.3	0.33	Pharmaceutical tablets	(Xu et al., 2009)
Nano-Au/PG/CPE	CV, DPASV	0.05–70	0.01	Commercial tablets	(Zhang et al., 2010b)
Graphene/GCE	CV, SWV	0.1–20	0.023	Commercial tablets, Plasma	(Kang et al., 2010)
SWNT modified EPPGE	CV, SWV	0.005–1.000	0.003	Human urine	(Goyal et al., 2010)
MWCNTs/GCE	CV, DPV	0.1–22 26–340	0.029	Human serum and urine Pharmaceutical tablet	(Babaei et al., 2011a)
Graphite oxide/GCE	CV, DPV	0.165–26.5	0.04	Pharmaceutical tablets	(Song et al., 2011)
MWCNT–ACS/GCE	SWV	0.05–2.0	0.05	Pharmaceutical tablets	(Lu & Tsai, 2011)
MWNTs-CHT/GCE	CV, DPV	2.0–250.0	0.16	Human serum and urine	Babaei et al. (2011b)
AuNPs/SDS-LDH/GCE	CV, DPV	0.5–400	0.13	Human serum	(Yin et al., 2011)
Nafion/TiO2–graphene/GCE	CV, DPV	1–100	0.21	Commercial tablet	(Fan et al., 2011)
ETPGE	ATSDPV	0.05–2.5 2.5–15.0	0.0025	Human blood serum	(Özcan & Şahin, 2011)
SWCNT/CCE	CV	2.9–100	0.48 0.62	Commercial tablet Syrup	(Habibi et al., 2011)
MWCNT/CCE					
BDDE	CV, SWV	0.4–100	0.21	Human urine samples	(Švorc et al., 2012)
AuNP-PGA/SWCNT	CV, DPV	8.3–145.6	1.18	Pharmaceutical tablets	(Bui et al., 2012)

Sensors [a]	Detection method	Linear range (μM)	Detection limit (μM)	Sample	Ref.
SWCNT–GNS/GCE	CV, DPV	0.05–64.5	0.038	Human serum	(Chen et al., 2012b)
MIPs/GNPs/MWNTs/GCE	EIS, DPV	0.08–50	0.04	–	(Zhu et al., 2013)
MIP micelle/amphiphilic photo-crosslinkable copolymer	CPE, CV, DPSV	1–4000	0.33	Pharmaceutical tablets	(Luo et al., 2013a)
MIP micelles	CV, DPV	10–8000	1.0	Commercial tablets, urine	(Wang et al., 2013c)
MIP/PPD/GCE	CV, SWV	0.0007–3333	0.2	Pharmaceutical tablets	(Pontie et al., 2014)

[a] The abbreviations are explained in Table 18

Table 18: The abbreviations included in Table 17

Abbreviation	Details
MIP-BAW	Molecularly Imprinted Polymer-Bulk Acoustic Wave
CV	Cyclic Voltammetry
SWV	Square Wave Voltammetry
DPV	Differential Pulse Voltammetry
AdsSV	Adsorptive Stripping Voltammetry
ACV	Alternating Current Voltammetry
DPASV	Differential Pulse Adsorptive Stripping Voltammetry
ATSDPV	Adsorptive Transfer Stripping Differential Pulse Voltammetry
CPE	Controlled Potential Electrolysis
DPSV	Differential Pulse Stripping Voltammetry
EIS	Electrochemical Impedance Spectroscopy
MIP-CFME	Molecular Imprinted Polymer-Carbon Fibre Microcylinder Electrode
C60/GCE	C60/Glassy Carbon Electrode
PANI-MWCNTs	Polyaniline–Multi-Walled Carbon Nanotubes
C–Ni/GCE	Carbon-coated Nickel Magnetic Nanoparticles Modified Glassy Carbon Electrodes
MIP-PGE	Molecular Imprinted Polymer-Pencil Graphite Electrode
SWCNT–DCP/GCE	Single-Wall Carbon Nanotube–Dicetyl Phosphate/Glassy Carbon Electrode
PAY/nano-TiO2/GCE	Poly(acid yellow 9)/Nano-TiO_2 coated GCE
TCPE/CILE	Traditional Carbon Paste electrode/Carbon Ionic Liquid Electrode
MWCNT–BPPGE	Multi-walled Carbon Nanotube-Basal Plane Pyrolytic Graphite Electrode
GNMCPE	Gold Nanoparticle Modified Carbon Paste Electrode
Nano-Au/PG/CPE	Nano-gold/Poly Glutamate/Carbon Paste Electrode

Abbreviation	Details
SWNT modified EPPGE	Single-Walled Carbon Nanotubes modified Edge Plane Pyrolytic Graphite Electrode
MWCNT–ACS/GCE	Multi-walled Carbon Nanotube-Alumina-Coated Silica/Glassy Carbon Electrode
MWNTs-CHT/GCE	Multi-walled Carbon Nanotube/Chitosan/Glassy Carbon Electrode
AuNPs/SDS-LDH/GCE	Gold Nanoparticles/Layered Double Hydroxide Sodium Modified with Dodecyl Sulphate/Glassy Carbon Electrode
SWCNT/CCT	Single-Wall Carbon Nanotube/Carbon-Ceramic Electrode
MWCNT/CCT	Multi-walled Carbon Nanotube/Carbon-Ceramic Electrode
ETPGE	Electrochemically Treated Pencil Graphite Electrode
BDDE	Boron-doped Diamond Electrode
AuNP-PGA/SWCNT	Gold Nanoparticle-Poly glutamic acid/Single-Wall Carbon Nanotube
SWCNT–GNS/GCE	Single-Wall Carbon Nanotube-Graphene Nanosheet/Glassy Carbon Electrode
MIPs/GNPs/MWNTs/GCE	Molecular Imprinted Polymers/Gold Nanoparticles/Multi-walled Carbon Nanotube/Glassy Carbon Electrode
MIP/PPD/GCE	Molecular Imprinted polymer/p-Phenylenediamine/Glassy Carbon Electrode

4.8.3 Other analytical methods

Several additional analytical techniques have been employed to determine acetaminophen such as HPLC (Ravisankar et al., 1998), capillary electrophoresis (Zhao et al., 2006) and chemiluminescence (Easwaramoorthy et al., 2001). As mentioned in the Section 2.5.3, these methods have several drawbacks, which make them unsuitable for routine analysis (Goyal et al., 2010; Luo et al., 2013a). In comparison with these methods, the fluorescence probe method is an alternative approach due to significant advantages including easy operation, fast and simple experimental process, low cost and high sensitivity for different fluorescent emission wavelengths of different molecular structures (Li et al., 2009a).

Table 19 summarises and compares the linear ranges and detection limits for the detection of acetaminophen achieved by various analytical methods.

Fatta et al. (2007) reviewed several advanced analytical methods that quantify pharmaceuticals at trace concentrations in water and waste water matrices. Different alternative methods and materials of extraction, derivatization and clean-up have been investigated to minimise matrix effects during GC-MS, GC-MS/MS, LC–MS or LC–MS/MS analysis.

Table 19: Comparison of the sensitivity of various analytical methods for acetaminophen

Method	Detector	Linear range (μM)	LOD (μM)	LOQ (μM)	Reference
Second derivative UV spectro-photometry	–	33.1–264.6	–	–	(Milch & Szabo, 1991)
Flow injection – Fourier-transform infrared spectrometry (FT-IR)	Temperature-stabilised fast recovery deuterated triglycine sulphate detector	10.6–31.7	1.32	–	(Bouhsain et al., 1996)
Flow injection – FTIR	Photoconductive narrow-band mercury–cadmium-telluride detector	48.0–960.0	–	–	(Ramos et al., 1998)
Supercritical fluid chromatography	UV-Vis detector	1.98–132.3	0.66	–	(Patil et al., 1998)
Ion chromatography	UV detection	3.3–46.3	0.4	–	(Pérez & Bello, 1999)
Capillary electrophoresis	UV detection	0.5–100	0.25	1.0	Bohnenstengel et al. (1999)
Flow injection-solid phase spectrophotometry	Spectrophotometric detector	16.5–264.6	0.69	–	(Medina et al., 1999)
HPLC	Variable visual/UV wavelength detector	10.3–1323.1	0.33	0.99	(Brunner & Bai, 1999)
Micellar electrokinetic chromatography	–	1720–3440	3.31	–	(Haque & Stewart, 1999)
Flow-through opto sensor	UV detection	3.31–53.0	0.15	–	(Cañada et al., 2000)
High-performance capillary electrophoresis	Amperometric detection	1–100	0.5	–	(Yang et al., 2000)
Flow injection	Chemiluminescence detection with photomultiplier tube	0.025–0.25	0.01	–	(Easwaramoorthy et al., 2001)
RP-HPLC with post-column enzymatic derivatization	Fluorescence detection	0.5–20	0.02	0.07	(Meyer & Karst, 2001)
	UV-Vis detection		0.5	1.7	

SPE is the most popular, well-established sample preparation technique, with which the best sensitivity is obtained. Alternative techniques [e.g. solid phase microextraction (SPME) and liquid-phase microextraction] have been applied more recently due to several advantages compared to SPE in terms of speed, ease of sample handling and minimising solvent use. LOD ranges for paracetamol by different techniques are summarised in Table 20, from which it can be seen that LODs using GC–MS are slightly higher than those obtained with LC–MS/MS in recent studies.

Table 20: Analytical methods for the determination of paracetamol in water and waste water

Detection	Extraction method	Linear range ($ng \cdot L^{-1}$)	LOD ($ng \cdot L^{-1}$)	LOQ ($ng \cdot L^{-1}$)	Sample	Ref.
GC–MS	SPME fibre coating: polyacrylate, carbowax-divinylbenzene, poly(dimethyl)-siloxane-divinylbenzene and C18	–	10 000	–	Ground and river water	(Möder et al., 2000)
HPLC–ESI-MS/MS (reverse phase)	SPE (Phenomenex Strata X as a stationary phase)	50–45 000	50	–	Sewage effluents and surface waters	(Hilton & Thomas, 2003)
GC–MS	SPE (LiChrolut ENV+) (Jones Chromatography, Mid Glamorgan, UK)	52–290	–	2–4	Surface waters	(Bound & Voulvoulis, 2006)
LC–MS/MS	SPE (Oasis HLB)	500–29 000	3.8–47	–	Hospital effluent waste water	(Gómez et al., 2006)
HPLC-ESI-MS/MS (reverse phase)	SPE (Strata X columns)	11–69,570	50	–	Waste water effluent and surface waters	(Roberts & Thomas, 2006)
GC–MS/MS	SPE (Oasis HLB sorbent)	100–1000	16	55	Hospital effluent	(Gómez et al., 2007)
HPLC-MS/MS (reverse phase)	SPE (Strata^{TM} X)		0.42	1.39	Natural and waste water	(Nebot et al., 2007)
GC–MS (reverse phase)	SPE (two-layer disks consisting of C18 and poly-(styrene divinylbenzene) copolymer (SDB-XC))	24.7–65.2	–	–	River waters	(Zhang et al., 2007)

GC–MS = Gas chromatography–mass spectrometry

HPLC-MS/MS = High-pressure liquid chromatography–tandem mass spectrometry

ESI = Electrospray ionization

SPME = Solid phase microextraction

SPE = Solid phase extraction

4.9 Conclusion

The reviewed literature shows that QD-based fluorescence sensors can offer the sensitivity required to analyse target ECPs in water systems. There is, however, a need for more research to be done to improve their selectivity towards target analytes. In many sensors, more than one analyte could give a fluorescence signal, and selectivity was based on which analyte gave more intensity. This, however, can be a limitation for application in complex environmental samples where there could be numerous compounds that are structurally similar to the analyte. Thus, selectivity approaches that incorporate MIPs, for example, have potential for these applications, or alternatively, the sensors should be employed to indicate the presence of a class of compounds (such as PAHs).

PAHs are ubiquitous environmental pollutants, which arise from the combustion of organic fuels. They have potential human health effects including carcinogenicity and PAHs have been found in South

African water systems at concentrations ranging from 0.1–52 $\mu g \cdot L^{-1}$ (Chimuka et al., 2015). The reviewed literature shows that QD fluorescence sensors could be a viable detection method for PAHs because of the low detection limits that can be achieved.

Most of the fluorescence sensors for pesticide applications have focused on organophosphate pesticides because of their inhibiting effects on enzymes that are used to make biosensors. Limited work has focused on organochlorine pesticides, like atrazine and terbuthylazine, which are both widely used in agriculture in South Africa. These two pesticides have been identified as contaminants of emerging concern in South African water systems (Odendaal et al., 2015), hence they should be included as targets for fluorescence sensor development. Other pesticides such as glyphosate are not routinely analysed in local laboratories because of difficulties arising from their chemical instabilities. Thus, the potential for fluorescence sensors as an alternative analytical technique to target such pesticides should be investigated.

The PPCPs have received much attention in environmental research. In spite of the fact that they exist at low concentrations, there is still a lot of concern regarding chemical persistence, microbial resistance, and synergistic effects of the numerous PPCPs present (Daughton & Ternes, 1999). Triclosan is a synthetic, non-ionic, broad spectrum antimicrobial agent that has been used extensively for more than 20 years. It is a relatively stable, lipophilic compound that has been detected in sewage sludge, discharge effluent, receiving surface waters and sediments (Hua et al., 2005). Although triclosan is sparingly soluble in water (McAvoy et al., 2002), and waste water treatment processes can remove 58–99% of triclosan (NICNAS, 2009), environmental concentrations of 1.4–40 000 $ng \cdot L^{-1}$ have been found in surface water (SCCS, 2010). It is thus important to develop sensitive and selective methods to detect the environmental fate of triclosan. No publications were found for the determination of triclosan using semiconductor QDs with the aid of fluorescence detection, although a great number of different methods have been reported for the detection and quantification of triclosan, including nanomaterial-based electrode systems with CV detection and MIPs that can be used for a broader linear range of triclosan in water samples.

Acetaminophen is one of the most extensively used analgesic and antipyretic drugs that can reduce fever and relieve moderate pain. It has been recognised as one of 95 contaminants in US streams (Kolpin et al., 2002). It is pervasive in the natural environment and it can easily accumulate in surface water, drinking water and waste water. Thus efficient, sensitive and simple analytical methodologies are of importance for the determination of acetaminophen. Although chemical oxidation processes can be used for the treatment of acetaminophen in waste water, harsh reaction conditions, high operational cost and the generation of secondary pollutants restrain the use thereof. Acetaminophen has been found at levels up to 10 000 $ng \cdot L^{-1}$ in surface water (Daughton & Ternes, 1999). A multitude of techniques have been developed to determine acetaminophen, including two semiconductor QD fluorescence-based methods.

Most of the current probes for the ECPs investigated function as solutions of the sensing materials. There is therefore a need for more research into the immobilisation of these nanoparticles to provide solid reusable devices.

This review highlighted the different detection methods for the determination of a number of target ECPs. Conventional methods for detecting these compounds are generally time-consuming, expensive and labour-intensive. QD-based detection systems are sparking a revolution in the field of environmental safety and analysis. They have the potential to be reliable tools for the screening of ECPs because of their high selectivity, sensitivity, simplicity, rapidity and high resistance to chemical degradation. They also enjoy many advantages over conventional methods in terms of minimum sample volume, cost and analytical time. Importantly, QDs have the potential to detect their targets accurately and selectively based on their photo physical and optical properties.

5 QUANTUM DOT SYNTHESIS AND CHARACTERISATION

By Hanieh Montaseri[9], Sifiso Nsibande[8], Oluwasesan Adegoke[10] and Patricia Forbes[8]

5.1 Introduction

5.1.1 Introduction to the synthesis of CdSe/ZnS QDs

With respect to obtaining high quality semiconductor QDs suitable for a wide range of chemical and biological applications, the hot-injection organometallic synthetic route is the most effective. The reason is due to the flexibility involved in tuning the optical properties of the QDs to eliminate as much as possible the presence of surface defects that diminishes the optical properties of the QDs. Among the classes of conventional Cd-based QDs, CdSe/ZnS core/shell QDs is the most popular, due to its better optical properties than the parent core and its low toxicity in the presence of the non-toxic ZnS shell layer. They have been synthesised at high temperature in organic media and stabilised with hydrophobic capping agents including TOPO and oleic acid (OA) (Dabbousi et al., 1997; Hines & Guyot-Sionnest, 1996; Sapsford et al., 2006; Xie et al., 2005).

A mixture of trioctylphosphine (TOP) and TOPO is the typical ligand system for most semiconductor QDs made from elements of groups II–VI and III–V such as CdSe and InP (Micic et al., 1994; Murray et al., 1993). These ligands not only provide all-important solubility in common organic solvents such as hexane and toluene, but they also serve as agents for reducing the unwanted electronic defects associated with dangling bonds or surface defect states. OA-capped CdSe/ZnS QDs can be suspended in many non-polar solvents such as chloroform, dichloromethane, toluene and hexane.

As these QDs have no tendency to be soluble in aqueous media, three main techniques have been developed to alter the surface of the QDs to enhance their water solubility. The first and most successful method for surface modification is ligand exchange reactions to replace TOPO or OA with hetero-bifunctional ligands such as mercaptocarbonic acids and thiol-terminated ligands, in which the mercapto or thiol end binds onto the QDs surface while the carboxyl moiety establishes water solubility (Chan et al., 2002; Chan & Nie, 1998). Pong et al. (2008) reported that free thiols are not capable of binding onto the ZnS surface. So, in order to enhance the reactivity of the thiol, the mixture must be dehydrogenated at the thiolic sulphur in basic environments which means that the bound state must be a thiolate.

In a second method, a cross-linked silica shell, which has been functionalised with polar groups, replaces the TOPO shell (Bruchez et al., 1998; Gerion et al., 2001). The third approach refers to encapsulation of TOPO-capped QDs with amphiphilic copolymers or phospholipids (Pellegrino et al., 2004; Wu et al., 2003). Even though the silica shell or encapsulation by a polymer/phospholipid provides good water solubility and quantum yield, they significantly increase the QD particle size and shell thickness, so they restrain the application of QDs in FRET processes because of the limitation in terms of proximity of acceptor and QD core. Short-chain mercaptocarbonic ligands are suitable for FRET and due to the thin barrier around the QDs they can be used in ion sensors based on fluorescence transduction changes (Chen & Rosenzweig, 2002; Jin et al., 2005).

Replacing TOPO or OA with mercapto acid ligands has posed some problems such as reduced quantum yield and poor colloid stability of the QDs in water (Algar & Krull, 2007; Gill et al., 2005; Kloepfer et al., 2005; Stsiapura et al., 2006). Gill et al. (2005) reported that when CdSe/ZnS QDs were functionalised with mercaptopropionic acid, quantum yields reduced by 50% (Stsiapura et al., 2006) and when a mixture of mercaptoacetic acid and mercaptosuccinic acids were used as the ligand

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exchanger, quantum yield diminished two times. The other problem is poor colloidal stability, which means that the nanoparticles do not tend to stay individually dispersed but form aggregates because energy acceptors are located deep within the aggregates which cannot be approached to close proximity (Pong et al., 2008).

Wang et al. (2007a) established a new strategy to allow ZnS shell formation and mercaptopropyl acid (MPA) functionalisation to be carried out simultaneously for the synthesis of MPA-capped CdSe/ZnS QDs. The PL intensity of the core-shell QDs can be preserved by this method. Generally, this finding proves that in ligand exchange reactions, the loss of quantum yield stems from changes in the structure of CdSe/ZnS, and not from the effect of mercapto-carbonic acids or thiol-terminated capping ligands. Theoretically, it is feasible to improve the ligand exchange reaction method and find the appropriate reaction conditions to eradicate the loss of quantum yield during ligand exchange.

As mentioned above, free thiols have no tendency to bind onto ZnS shells. So, the reactivity of the thiol must be enhanced by removing the thiolic hydrogen. In an in-depth investigation by Pong et al. (2008), they improved procedures to functionalise CdSe/ZnS QDs with MPA. They used mild reaction conditions (tetramethylammonium hydrogen) at room temperature to dehydrate thiolic hydrogen. Not only was the quantum yield of QDs increased, but the colloidal stability was also improved to four months. In fact, the bonding strength of the Zn-S_{thiol} and S-S_{thiol} were 46.5 kcal·mol^{-1} and 25.1 kcal·mol^{-1}, respectively and alkyl thiols cannot directly interact with the hexagonal wurtzite structure of the ZnS shell. It was therefore crucially important to control the surface coverage of the ligands to preserve quantum yield. Table 21 lists the stabilising agents, particle size, and precursors for the synthesis of CdSe/ZnS QDs and also target analytes.

Table 21: Requirements for synthesising CdSe/ZnS QDs

Particle size (nm)	Precursors [a]	Stabilising agent	Target	Ref.
1.2 ± 0.6	Me_2Cd, Se, TOPO, TOP, Me_2Zn, $(TMS)_2S$	–	NA	(Hines & Guyot-Sionnest, 1996)
2.3–5.5	$CdMe_2$, Se, TOPO, TOP, $ZnEt_2$, $(TMS)_2S$	–	NA	(Dabbousi et al., 1997)
6–17 (Silica) 5–12 (MPA)	TOPO, MPS, APS, TMAH	Silica, MPA	NA	(Gerion et al., 2001)
5 ± 0.2	Commercial	Histidine and *N*-acetyl-cysteine	NA	(Ai et al., 2007)
<10	$Cd(OAc)_2$, HDA, TOPO, TOP, Se, zinc ethyl xanthate, TBP	MPA, ME, AEE, L-cys, Cys-dipeptide	NA	(Murcia et al., 2008)
–	CdO, TOPO, TOP, Se, $ZnEt_2$, $(TMS)_2S$	L-cys, D-cysteine	Carnitine enantiomers	(Carrillo-Carrión et al., 2009)
5	CdO, Se, ODE, OA, S, ZnO, ODA	PMMA	Paeonol	(Dong et al., 2011)
2.9 ± 0.3 5.6 ± 0.6	Commercial	Methyl ester N-acetyl-l-cysteine (CysP)	Aryl propionic acids, ketoprofen, naproxen, flurbiprofen, ibuprofen	(Delgado-Pérez et al., 2013)
~5.5	TOPO, cysteamine, DMAET, EDAC	L-cys, PEG then Ce6	NA	(Martynenko et al., 2013)
10–12	CdO, ODE, OA, TOP, Se, Me_2Zn, $(TMS)_2S$	L-cys, TGA, MPA, MSA, MUA	NA	(Rahman et al., 2014)
2–3.5	Se, $NaBH_4$, $CdCl_2{\cdot}2.5H_2O$, L-Cys, $ZnCl_2$	GSH	Biomedical use on chicken embryo	(Kuzyniak et al., 2014)

Table 22: The abbreviations included in Table 21

Abbreviations	Details
Me_2Cd	Dimethyl cadmium
$(TMS)_2S$	Bis(trimethylsilyl) sulphide
TOPO	Trioctylphosphine oxide
TOP	Tri-*n*-octyl phosphine
$ZnEt_2$	Diethylzinc
MPS	Mercaptopropyltris(methyloxy)silane
APS	Aminopropyltris(methyloxy)silane
TMAH	Tetramethylammonium hydroxide
MPA	Mercaptopropionic acid
$Cd(OAc)_2$	Cadmium acetate
HDA	Hexadecylamine
TBP	Tributylphosphine
ME	Mercaptoethanol
AEE	Aminoethoxyethanol
L-cys	L-cysteine
CdO	Cadmium Oxide
ODE	1-octadecene
OA	Oleic acid
ZnO	Zinc oxide
ODA	Octadecylamine
PMMA	Polymethylmethacrylate
DMAET	2-(dimethylamino)ethanethiol
EDAC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
PEG	Polyethylene glycol
Ce6	Chlorin e6
Me_2Zn	Dimethylzinc
TGA	Thioglycolic acid
MPA	Mercaptopropionic acid
MSA	Mercaptosuccinic acid
MUA	Mercaptoundecanoic acid
$NaBH_4$	Sodium borohydride
$CdCl_2{\cdot}2.5H_2O$	Cadmium chloride hemi (pentahydrate)
$ZnCl_2$	Zinc chloride
GSH	Glutathione

5.1.2 Introduction to the synthesis of GQDs

PAHs are potentially harmful pollutants emitted into the environment from a range of sources as a consequence of combustion processes (Chimuka et al., 2015). These compounds can occur in natural waters through point and non-point source discharges to surface, ground and drinking water (Manoli & Samara, 1999). There are serious concerns around exposure to PAHs as some of them have been acknowledged as probable human carcinogens (Kim et al., 2013b), hence there is need for continuous environmental monitoring of these compounds.

Significant research is being done on semiconductor-based QDs made from inorganic metal precursors. Although these QDs have excellent fluorescence properties – and can have versatile applications because of surface modifications – the use of heavy metals like cadmium in their core has raised health concerns. In recent years, GQDs have emerged as possible alternative to semiconductor QDs. GQDs can be regarded as extremely small, zero-dimensional, pieces of graphene and have properties of graphene, which have quantum confinement and edge effect properties similar to carbon dots (Li et al., 2013). These QDs have certain advantageous properties over semi-conductive QDs, including higher photostability, biocompatibility, large surface area, better surface grafting using π-π conjugation and surface groups, and they have very low toxicity (Shen et al., 2012a). Because of their low toxicity, they have also been widely used for biological applications (Zheng et al., 2015). Thus, they have recently found extensive application in designing sensing probes (Benítez-Martínez & Valcárcel, 2015; Lin et al., 2014; Shen et al., 2012a; Sun et al., 2013). Their application in analytical chemistry has mostly been towards metal ions sensing (Ju & Chen, 2015) with only a few studies reporting their use for organic pollutant detection such as for pentachlorophenol (Liu et al., 2014a), trinitrotoluene (Fan et al., 2012) and the pesticide tributyltin (Zor et al., 2015).

The fluorescence properties of GQDs arise from the radiative recombination of electron-hole (e-h) pairs in sp^2 aromatic carbon sites. This is because graphene has infinitely large Bohr diameters (distance between electron and hole) and therefore its fragments, i.e. GQDs, of any size, exhibit quantum confinement effects (Zhu et al., 2015). The π-π* electronic transitions are confined.

GQDs can be prepared using two approaches, namely, the top-down approach or the bottom-up approach. The top-down approach involves cutting of large macroscopic carbon materials (e.g. graphite, carbon fibres, GO, metal-organic frameworks, etc) into small GQDs pieces, usually through harsh oxidations treatments. On the other hand, the bottom-up approach involves GQDs formation from molecular or atomic carbon precursors (e.g. chlorobenzene, acetic acid, glucose, and even from PAHs) through controlled reactions to obtain desired GQDs sizes (Benítez-Martínez & Valcárcel, 2015; Shen et al., 2012a). GQDs can be doped, usually with nitrogen, to improve their PL properties. Studies by Qu et al. (2014) showed that N-doped GQDs can have an improved quantum yields of up to 94%.

In this part of the report we are therefore reporting on progress and preliminary results on the syntheses and characterisation of sensor materials for targeted PAHs. We synthesised GQDs using the top-down approach using graphite as a starting material (Figure 34) and studied the properties and possible application of the GQDs as sensors for PAHs. Graphene materials have previously been shown to interact strongly with PAHs due to π-π interactions (Wang et al., 2015). We therefore explore this interaction on GQD surfaces to develop a probe for PAH detection.

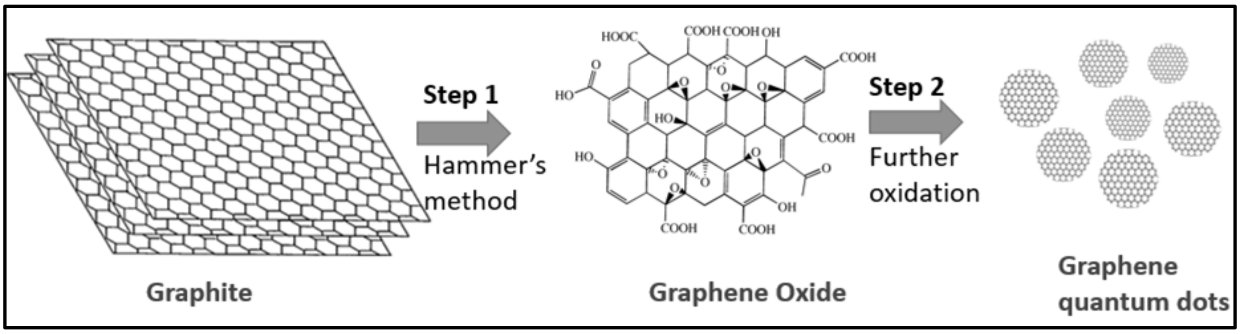


Figure 34: Schematic representation of synthesis of GO from graphite with further oxidation to produce GQDs

5.2 Experimental

5.2.1 Materials and methods for synthesis of CdSe/ZnS

TOPO, octadec-1-ene (ODE), zinc oxide, cadmium oxide, OA, sulphur powder, GSH, L-Cys and N-acetyl-L-cys (NAC) were purchased from Sigma-Aldrich. Hydrogen peroxide, sulphuric acid (H_2SO_4), methanol, chloroform, acetone, selenium powder (Se) and potassium hydroxide (KOH) were purchased from Merck. An ultrapure Milli-Q Water System was used for sample preparation.

5.2.1.1 Preparation of the precursors

In order to prepare the Se precursor, 1.9 g of TOPO in 25 mL of ODE was heated up to 80°C to produce a clear solution then 0.3 g of Se powder was added and stirred continuously to generate the TOPSe solution. For the preparation of sulphur precursor, 1.9 g of TOPO in 30 mL of ODE, and 20 mL of OA was heated to 80°C and once a clear solution was formed, 0.16 g of S powder was added. Zinc precursor was prepared by dissolving 0.41 g of ZnO powder in 30 ml of ODE and 20 ml of OA.

5.2.1.2 Fabrication of the QDs

One-pot synthesis was carried out for the synthesis of the core/shell QDs according to published methods with some modifications (Adegoke & Forbes, 2016; Mekis et al., 2003). The one-pot approach can be realised only if both core and shell can grow controllably in the same reaction mixture. Briefly, in a three-necked flask fitted with a thermometer, refluxing condenser and septum, 1.3 g of CdO was added into a solution of 50 mL of ODE and 30 mL of OA and the solution was vigorously stirred to a temperature of ~280°C so as to form a colourless Cd-OA complex under inert atmosphere. Once the colourless complex solution was formed, a pre-mixed TOPSe solution was injected into the solution to allow for nucleation and growth of CdSe QDs. In order to passivate the alloyed core surface, ZnS shell was epitaxially grown on the core surface by adding zinc and sulphur precursors at a reduced temperature of 200°C. The QDs were purified by methanol to completely remove unreacted precursors.

5.2.1.3 Water solubilization of the QDs with L-cys, GSH and NAC as capping agents

Water solubilization of the QDs was achieved via ligand exchange reaction of the hydrophobic capping (OA and TOPO) with the hydrophilic thiol L-cys, GSH or NAC ligands. Typically, thiol–KOH methanolic stock solution was prepared separately by adding 2 g of L-cys, GSH or NAC to 3.0 g of KOH in 40 mL methanol with dissolution assisted by ultrasonication. The purified OA–TOPO-capped CdSe and CdSe/ ZnS QDs were re-dispersed in chloroform and the thiol–KOH methanolic solution was subsequently added followed by the addition of Millipore water separately to each QD solution.

The solutions were stirred for 1 h for effective separation of the organic phase from the aqueous phase. The water-soluble CdSe/ZnS-L-cys QDs were purified repeatedly via centrifugation with ethanol followed by acetone to completely remove all unreacted organic substituents. While, CdSe/ZnS-NAC and CdSe/ZnS-GSH QDs were purified as follows: Step 1: acetone; Step 2: chloroform; Step 3: acetone. The purified aqueous dispersible QDs were dried in a fume hood.

5.2.2 Materials and methods for synthesis of GQDs

PAH standards were purchased from Sigma-Aldrich. Hydrogen peroxide (H_2O_2), sulphuric acid (H_2SO_4), sodium nitrate ($NaNO_3$), hydrochloric acid (HCl), graphite powder, potassium hydroxide (KOH) and potassium permanganate (KMnO4) were purchased from Merck. An ultrapure Milli-Q Water System was used for water preparation.

5.2.2.1 Preparation of GO

GO was prepared using a modified Hammer's method, which we previously reported on (Adegoke & Forbes, 2016). Briefly, 2.5 g of graphite powder and 1.3 g of $NaNO_3$ were mixed in a 250 mL three-neck round-bottom flask containing 60 mL of H_2SO_4. This mixture was stirred continuously inside an ice bath to allow thorough dispersion of the graphite powder for about 30 min. Afterwards 7.5 g $KMnO_4$ was gradually added and the mixture was left stirring for 2 h until it turned greenish. The mixture was then removed from the ice and left to stir at room temperature (~27°C) resulting in a pasty light-brown mixture forming. The following day, the mixture was again placed in an ice bath and 75 mL Millipore water was slowly added under stirring for 2.5 h. The temperature of the solution was then raised to ~60°C and allowed to stir. On the following day, 25 mL of 30% H_2O_2 was added to the reaction mixture followed by centrifuging with 5% HCl and lastly centrifuging with Millipore water. The solid GO was dried in an oven at 65°C to give a dark grey powder with a yield of 5.8 g.

5.2.2.2 Synthesis of GQDs

GQDs were prepared from GO using a previously reported method with some modifications (Fan et al., 2015). 1.0 g of GO was added to 40 mL H_2SO_4 and the mixture was stirred in ice until the next day. $KMnO_4$ was then added and the mixture was allowed to stir at room temperature for about 1 h before raising the temperature to about ~60°C and heating it until the following day.

The reaction was quenched by adding ice cold water containing small amounts of H_2O_2 and was left at room temperature for about 2 days to allow for settling. The supernatant containing GQDs was carefully pipetted out and used as is (in solution form) for subsequent fluorescence studies.

5.2.3 Materials and methods for synthesis of CdSe, CdSeTe/ZnSe/ZnS and ZnSeS/ZnTe/ZnS QDs

5.2.3.1 Materials

TOPO, cadmium oxide, ODE, tellurium powder (Te), zinc oxide, zinc diethyldithiocarbamate (ZDC), L-cys, OA, zinc oxide (ZnO) and sulphur (S) were purchased from Sigma-Aldrich. Methanol, absolute ethanol, chloroform, acetone, selenium powder (Se), and potassium hydroxide (KOH) were purchased from Merck. An ultrapure Milli-Q Water System was used for sample preparation.

5.2.3.2 Precursors

Se precursor was prepared by dissolving 1.9 g of TOPO in 25 mL of ODE to produce a clear solution then adding 0.3 g of Se powder. This was then stirred continuously to give the TOPO-Se solution used as Se precursor. The sulphur precursor was prepared by dissolving 1.9 g of TOPO in 30 mL of ODE, and 20 mL of OA followed by addition of 0.16 g of S powder form TOPO-S precursor. Tellurium precursors (TOPO-Te) were prepared by dissolving 0.48 g of Te powder and 1.93 g of TOPO in 25 mL of ODE. Finally, the Zn precursor was prepared by dissolving 0.41 g of ZnO powder in 30 ml of ODE and 20 ml of OA.

5.2.3.3 Synthesis of CdSe and CdTeSe/ZnSe/ZnS

These two QDs were produced using the one-pot synthesis using reported procedures for the synthesis of (Adegoke & Forbes, 2016), with modifications. Briefly, 1.3 g of CdO was added into a solution of 50 mL of QDE and 30 mL of OA and the solution was vigorously stirred in a three-necked flask under argon atmosphere to a temperature of 260°C in order to form a colourless Cd-OA complex. To form CdSe QDs, the Se precursor was added to the solution and nucleation was allowed to take before harvesting the QDs.

To form CdTeSe/ZnSe/ZnS QDs, the TOPTe solution containing 0.48 g of Te and 1.93 g of TOPO in 25 mL of ODE was added into the solution and this was followed swiftly by the addition of a TOPSe solution containing 0.30 g of Se and 1.93 g of TOPO in 25 mL of ODE. Nucleation and growth of the alloyed core QDs was allowed to proceed for ~15 min after which a ZnO solution containing 0.41 g of ZnO dissolved in 20 mL of OA and 30 mL of ODE was injected into the growth solution and this was followed immediately by the addition of the TOPSe solution. This resulted in the overcoating of the ZnSe shell around the alloyed CdSeTe core surface. Passivation of the CdSeTe/ZnSe core/shell QDs with a ZnS shell was carried out by adding into the core/shell growth solution, 0.407 g of ZDC dissolved in 30 mL of ODE and 20 mL of OA. ZDC was employed as a single molecular precursor because it dissolves in solution to form ZnS metal sulphide. The CdSeTe/ZnSe/ZnS QDs were allowed to react for around 2 h.

For all QDs, the purification of the hydrophobic QDs was carried out using methanol followed by acetone. These were then converted into hydrophilic QDs via ligand exchange reaction using KOH, metholic-L-cys-H_2O solution.

5.2.3.4 Synthesis of ZnSeS/ZnTe/ZnS

Briefly, 1.3 g of ZnO was added to a mixture of 30 mL of OA and 50 mL of ODE and the solution was heated up to 260°C under a steady flow of Ar gas. 10 mL of TOPO-Se solution was added into solution followed by addition of 15 mL of the sulphur precursor. The solution was allowed to react for 30 min to allow for effective formation of the alloyed ZnSeS QDs.

To overcoat the alloyed ZnSeS core with a ZnTe shell, 10 mL of the Zn precursor was injected into the growth solution followed immediately by injection of a 10 mL mixture of TOPO-Te to generate the Te source. The growth solution was left to react for 1 h for effective passivation of the shell layer. The deposition of a ZnS shell around the alloyed ZnSeS/ZnTe surface was carried out by using ZDC as a single molecular precursor source to generate ZnS in solution. The QD nanocrystals were purified using methanol followed by ethanol and subsequently re-dispersed in chloroform for further use.

5.3 Apparatus

Fluorescence emission spectra were recorded on a Horiba Jobin Yvon FluoroMax-4 spectrofluorometer. Powder XRD patterns were analysed using a PANalytical X'Pert Pro powder diffractometer in θ-θ configuration with an X'Celerator detector, variable divergence and receiving slits with Fe filtered Co-Kα radiation ($\lambda = 1.789$Å). FT-IR analyses were performed using a Bruker Vertex 70V. Stereo-microscope analysis was employed using Zeiss Discovery V20 Stereo Microscope, Jena, Germany. High resolution scanning electron microscopy (HRSEM) measurements were taken with an FE-SEM (Zeiss Ultra Plus). Ultraviolet visible (UV-vis) absorption spectra were recorded using a Cary Eclipse (Varian) spectrophotometer.

5.4 Results and Discussion

5.4.1 Semiconductor QDs

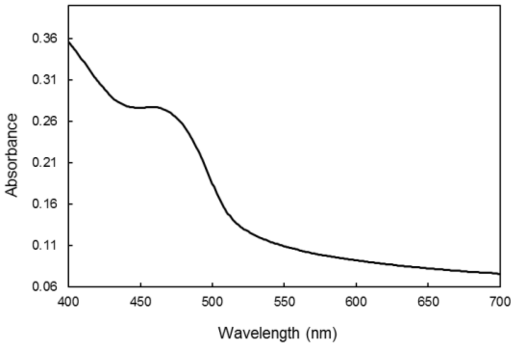
5.4.1.1 Absorption and PL emission characterisation of CdSe/ZnS-L-cys QDs

The absorption spectra of the synthesised water-soluble L-cys-capped CdSe and CdSe/ZnS QDs are shown in Figure 35(a) and Figure 35(b). A well-pronounced and broad excitonic peak at 457 nm and 515 nm and were obtained for the QDs respectively.

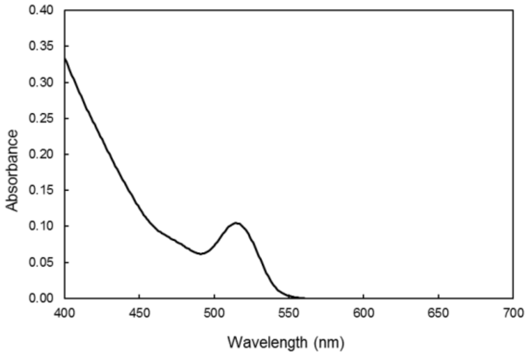
The surface passivation with a ZnS shell increased the size of core-shell QDs. It is important to note that the fluorescence enhancement of CdSe/ZnS QDs is because of the larger band-gap ZnS shell that effectively traps the electrons in the core (Figure 36(a).

Figure 36(b) shows a symmetric PL emission spectrum of water-soluble L-cys-capped CdSe and CdSe/ZnS QDs with a peak at wavelength of 569 nm and 598 nm, with the intensities of 5.0×10^5 and 1.5×10^7, while the estimated full width at half maximum (FWHM) were 37 nm and 48 nm for CdSe-L-Cys and CdSe/ZnS-L-cys respectively. The capping process has led to a significant modification of the CdSe as core and CdSe/ZnS as core/shell QDs.

The QDs exhibited a band edge type of PL emission, which is attributed to the elimination of deep trap emission mostly inherent in the red tail region of PL emission spectra of the QDs (De Geyter et al., 2010). It can also be seen that the PL emission of CdSe/ZnS-L-cys QDs had a red-shift of 29 nm compared to the parent CdSe-L-cys core and was also enhanced. The red-shift provides direct evidence of the presence of L-cys capping on the surface of the QDs and its influence on the PL emission spectra of the QDs. The size-dependent PL emission peak shift can generally be attributed to the quantum-size behaviour of semiconductor nanoparticles of size <10 nm (Geszke-Moritz & Moritz, 2013).



(a)



(b)

Figure 35: Absorption spectra of the water-soluble L-cys-capped CdSe (a) and CdSe/ZnS (b) QDs in Millipore water

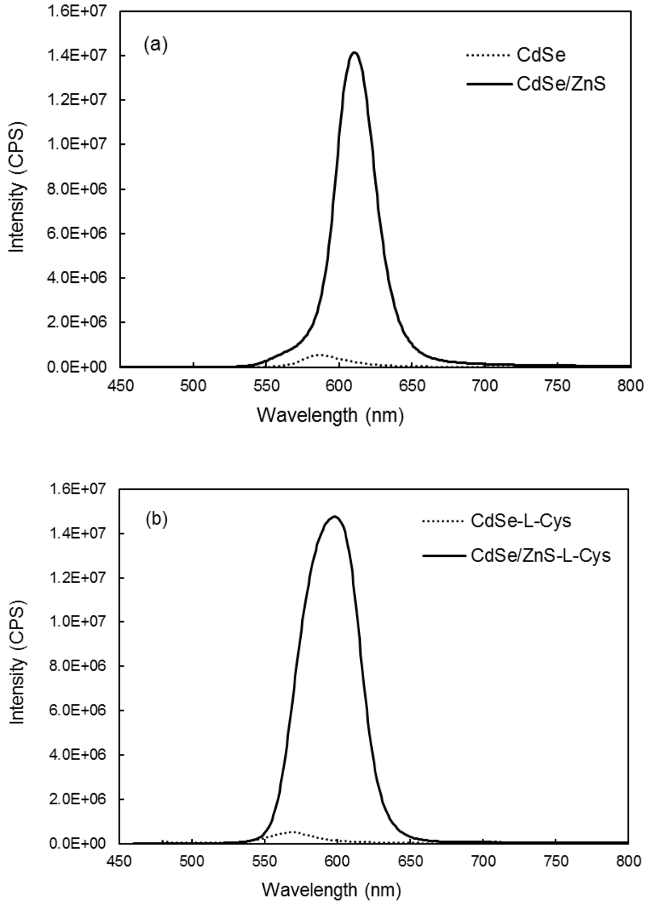


Figure 36: (a) PL emission of CdSe QDs (dotted line) and CdSe/ZnS QDs (solid line) before capping with L-cys at λ_{exc} 370 nm; (b) PL emission spectra of the water-soluble L-cys-capped CdSe QDs (dotted line) and L-cys-capped CdSe/ZnS QDs (solid line) measured in Millipore water at λ_{exc} 470 nm and 450 nm respectively

5.4.1.2 Absorption and PL emission characterisation of CdSe/ZnS-NAC QDs

Figure 37 illustrates the absorption spectra of the newly synthesised water-soluble NAC-capped CdSe/ ZnS QDs. A well-pronounced and broad excitonic peak at 521 nm was obtained for the QDs.

PL emission of CdSe QDs (dotted line) and CdSe/ZnS QDs (solid line) before capping with NAC at λ_{exc} 370 nm are illustrated in Figure 38(a). It can also be observed that the PL emission of CdSe/ZnS QDs had a red-shift of 25 nm compared to the CdSe and was also enhanced.

Figure 38(b) shows a symmetric PL emission spectrum of water-soluble CdSe/ZnS QDs at different excitation wavelengths. It is important to note that the emission wavelength did not shift with the change of excitation wavelength. Hence, the product is very pure and suitable for further study. Furthermore, emission wavelength of 607 nm with the intensity of 3.5×10^6 at excitation of 460 nm was selected for CdSe/ZnS-NAC, the estimated FWHM was also 31 nm.

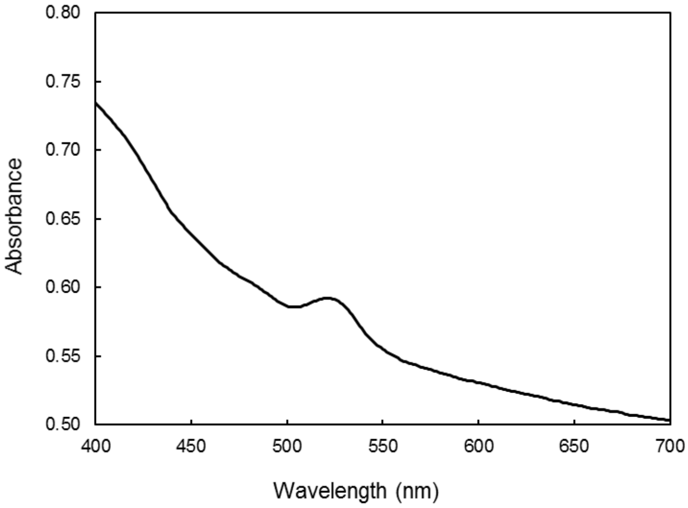


Figure 37: Absorption spectra of the water-soluble NAC-capped CdSe/ZnS QDs in Millipore water

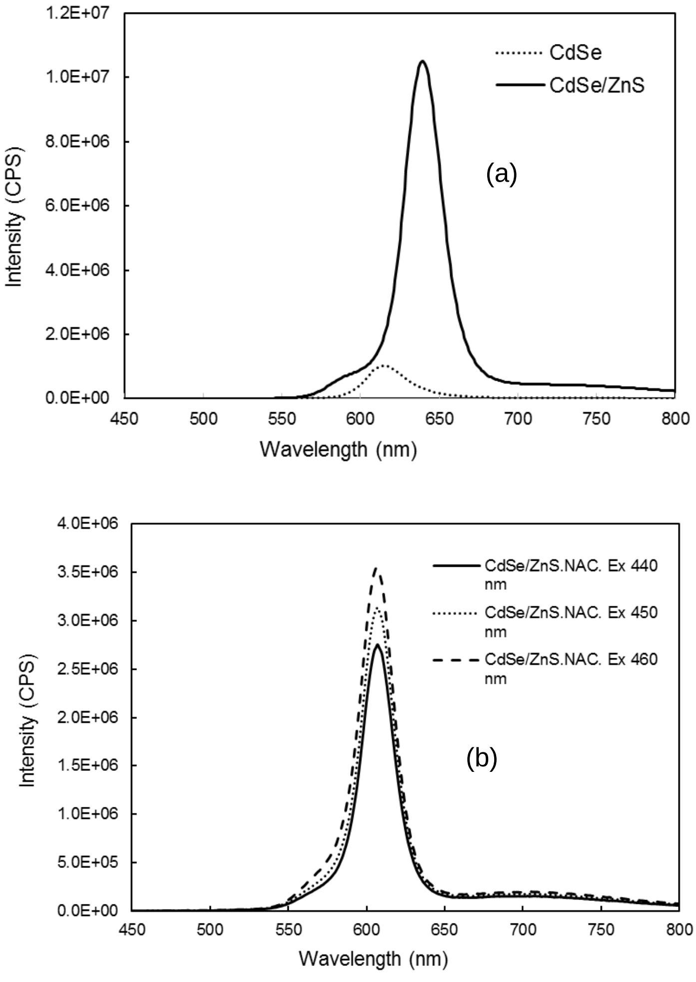


Figure 38: (a) PL emission of CdSe QDs (dotted line) and CdSe/ZnS QDs (solid line) before capping with NAC at λ_{exc} 370 nm; (b) PL emission spectra of the water-soluble NAC-capped CdSe/ZnS QDs measured in Millipore water at different excitation wavelength

5.4.1.3 *Absorption and PL emission characterisation of CdSe/ZnS-GSH QDs*

Figure 39 illustrates the absorption spectra water-soluble GSH-capped CdSe and CdSe/ZnS QDs. A well-pronounced and broad excitonic peak at 575 nm was obtained for the GSH-CdSe/ZnS QDs while CdSe-GSH has two excitonic peaks corresponding to 473 and 567 nm.

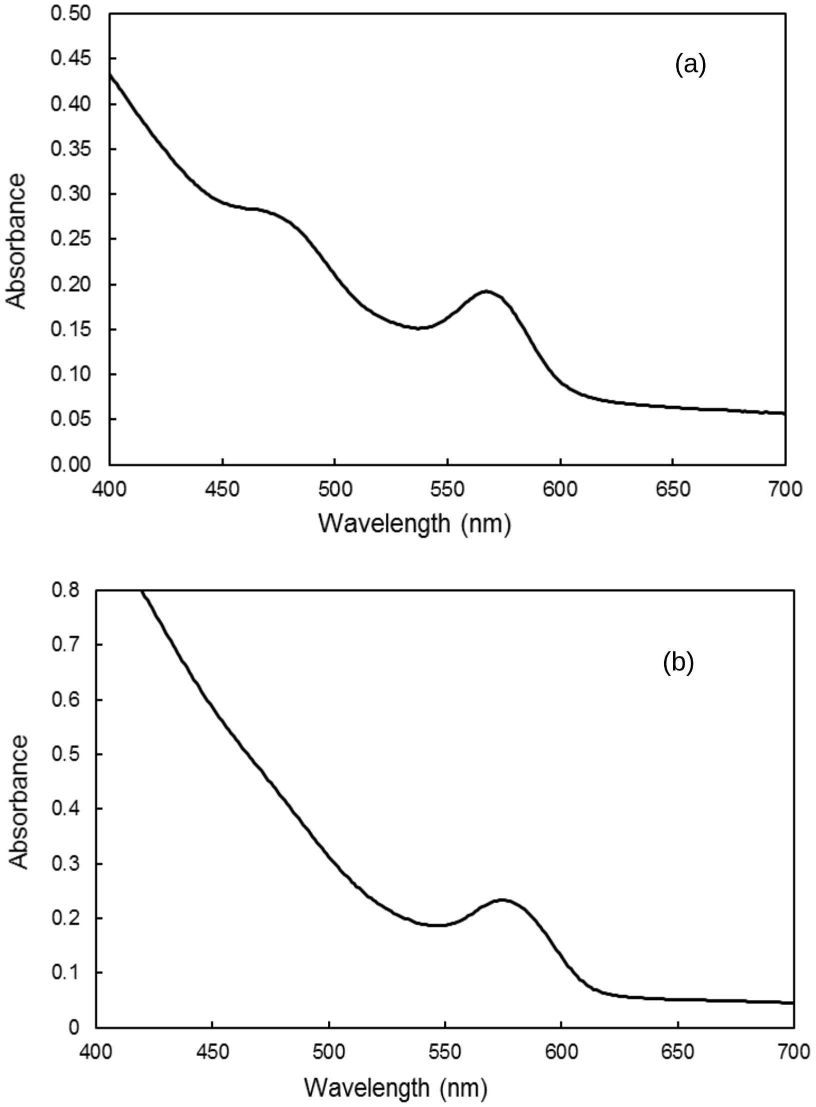


Figure 39: Absorption spectra of the water-soluble GSH-capped CdSe (a) and CdSe/ZnS (b) QDs in Millipore water

PL emission of CdSe QDs (dotted line) and CdSe/ZnS QDs (solid line) before capping with GSH at λexc 370 nm are illustrated in Figure 40(a). It can also be observed that the PL emission of CdSe/ZnS QDs had a red-shift of 21 nm compared to the CdSe and was also enhanced.

Figure 40(b) shows a symmetric PL emission spectrum of water-soluble-GSH-capped CdSe and CdSe/ ZnS QDs with a peak at wavelength of 576 nm and 605 nm, with the intensities of 8.5×10^5 and 1.2×10^7, while the estimated FWHM were 42 and 38 nm for CdSe-GSH and CdSe/ZnS-GSH respectively.

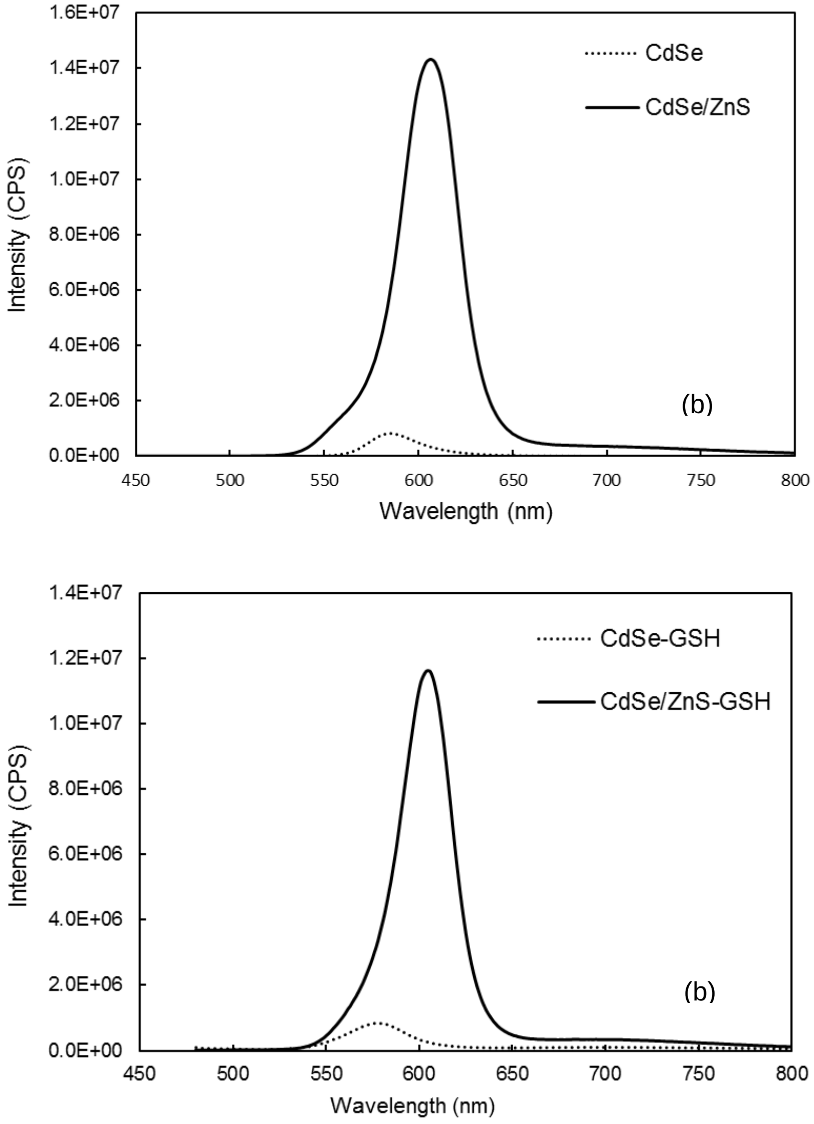


Figure 40: (a) PL emission of CdSe QDs (dotted line) and CdSe/ZnS QDs (solid line) before capping with GSH at λ_{exc} 370 nm; (b) PL emission spectra of the water-soluble GSH-capped CdSe QDs (dotted line) and GSH-capped CdSe/ZnS QDs (solid line) measured in Millipore water at λ_{exc} 470 nm

5.4.1.4 Powder XRD

Powder XRD is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions (Kumar et al., 2013).

CdSe/ZnS-L-cys QDs

Figure 41 compares the XRD patterns of CdSe-L-cys and CdSe/ZnS-L-cys QDs. A typical zinc blende crystal structure with planes at 111, 220 and 311 was obtained for CdSe/ZnS QDs with peaks at 31.55°, 52.71° and 62.74°.

The peaks for CdSe QDs were at 29.57°, 49.18° and 58.44° corresponding to 111. 220 and 311. This confirmed that it had a zinc blende crystalline structure. With respect to the 2θ position of the diffraction peaks, the diffraction peaks for alloyed CdSe/ZnS QDs are slightly shifted to higher Bragg angles in comparison to the alloyed CdSe core.

The broadening of the diffraction peak is due to the nano-sized dimensions of the nanocrystals.

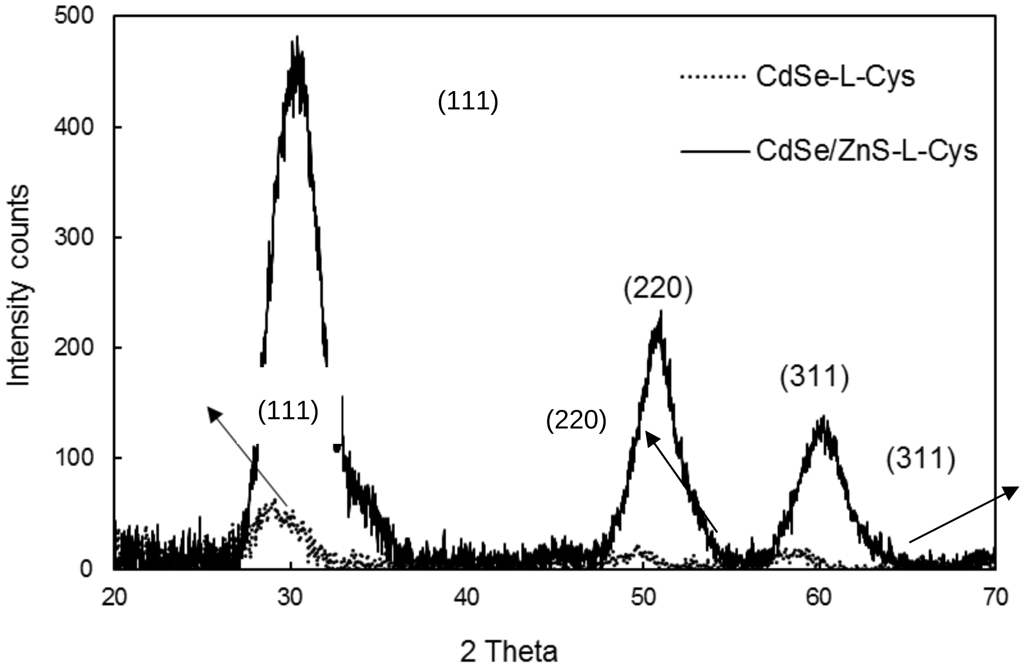


Figure 41: Powder XRD pattern of the CdSe L-cys and CdSe/ZnS-L-cys QDs

CdSe/ZnS-NAC QDs

XRD pattern of CdSe/ZnS QDs with NAC showed a zinc blende crystal structure with the three notable peaks at 31.6, 52.7, and 62.7 theta (Figure 42). The diffraction peaks are indexed to the scattering planes: 111, 220 and 311. Those sharp signals at 37.1, 40.3, 42.4, 55.8 and 66.8 theta are spurious signals and are not related to X-rays.

The peaks for CdSe QDs were at 29.6°, 49.2° and 58.4° corresponding to 111, 220 and 311. This confirmed that it had a zinc blende crystalline structure. ZnS can have two structures, zinc blende and wurtzite. The zinc blende structure is more thermodynamically favoured; however, because of the slow construction of wurtzite structures, both forms of ZnS can be found. They have 1:1 stoichiometry ratio of Zn to S. Wurtzite has a hexagonal structure, while zinc blende is cubic. The difference between wurtzite and zinc blende lies in the different arrangements of layers of ions.

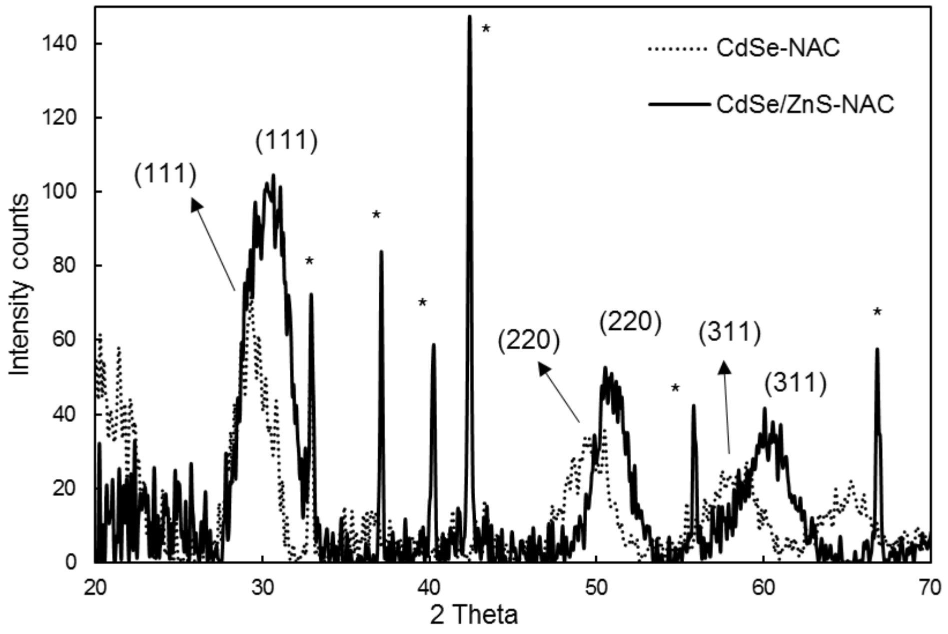


Figure 42: Powder XRD pattern of the CdSe-NAC and CdSe/ZnS-NAC QDs. A spurious signal is indicated by the asterisk (*)

CdSe/ZnS-GSH QDs

Figure 43 compares the XRD patterns of CdSe-GSH and CdSe/ZnS-GSH QDs. A typical zinc blend crystal structure with planes at 111, 220 and 311 was obtained for CdSe/ZnS QDs with peaks at 31.55°, 52.71° and 62.74°.

The peaks for CdSe QDs were at 31.57°, 53.74° and 59.63° corresponding to 111, 220 and 311. This confirmed that it also had a zinc blende crystalline structure. The diffraction peaks for alloyed CdSe/ZnS QDs are slightly shifted to higher Bragg angles in comparison to the alloyed CdSe core.

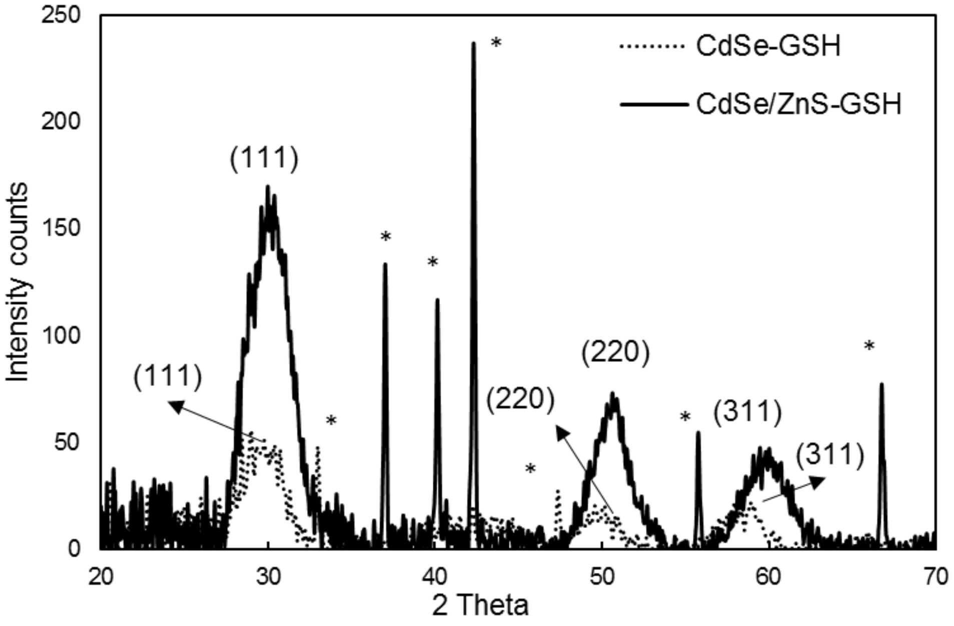


Figure 43: Powder XRD pattern of the CdSe-GSH and CdSe/ZnS-GSH QDs. A spurious signal is indicated by the asterisk (*)

5.4.1.5 FT-IR analysis

FT-IR analysis was carried out to characterise the functional groups on the surface of the materials.

CdSe/ZnS-L-cys QDs

FT-IR spectra of CdSe-L-cys, CdSe/ZnS-L-cys, annealed CdSe/ZnS-L-cys QDs and L-cys are depicted in Figure 47–Figure 47.

The –COO− group has strong and medium infrared absorption bands around 1550–1600 cm^{-1} and 1400 cm^{-1} respectively, while the absorption band around 3000–3500 cm^{-1} indicates the presence of hydroxyl groups.

The medium peak at 2900–3420 cm^{-1} shows $–NH_2$ group and the weak peak at 600–800 cm^{-1} indicates the C–S group. Moreover, carboxylic acid and amino groups are present on the surface of the L-cys-capped CdSe/ZnS QDs and L-cys, while the S–H group vibration (2550–2750 cm^{-1} wʋ S–H) is absent on the surface of the L-cys-capped CdSe/ZnS and CdSe QDs, which is attributed to the formation of covalent bonds between thiols and the surface of ZnS.

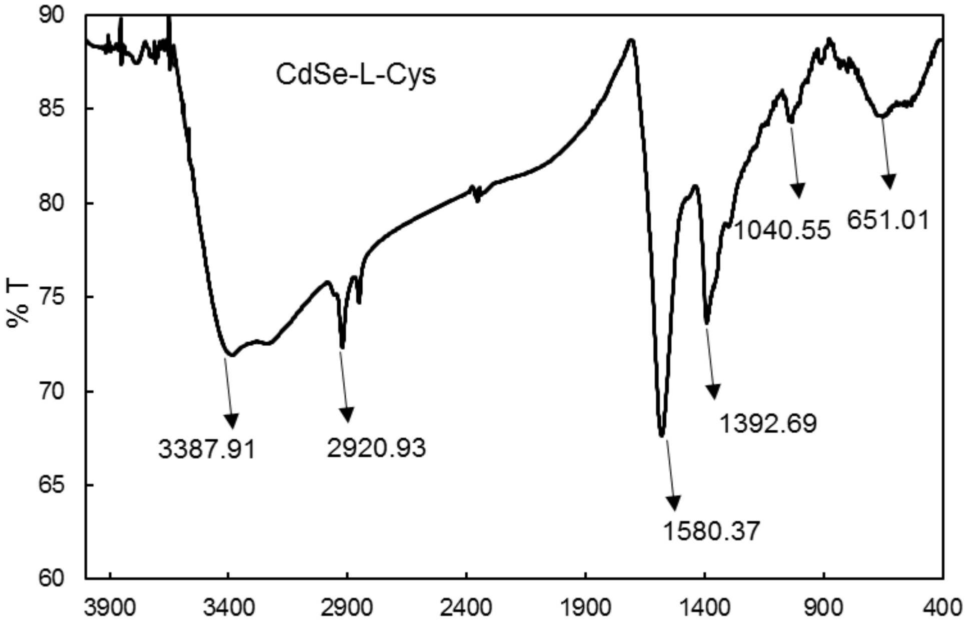


Figure 44: The FT-IR spectra of CdSe-L-cys QDs

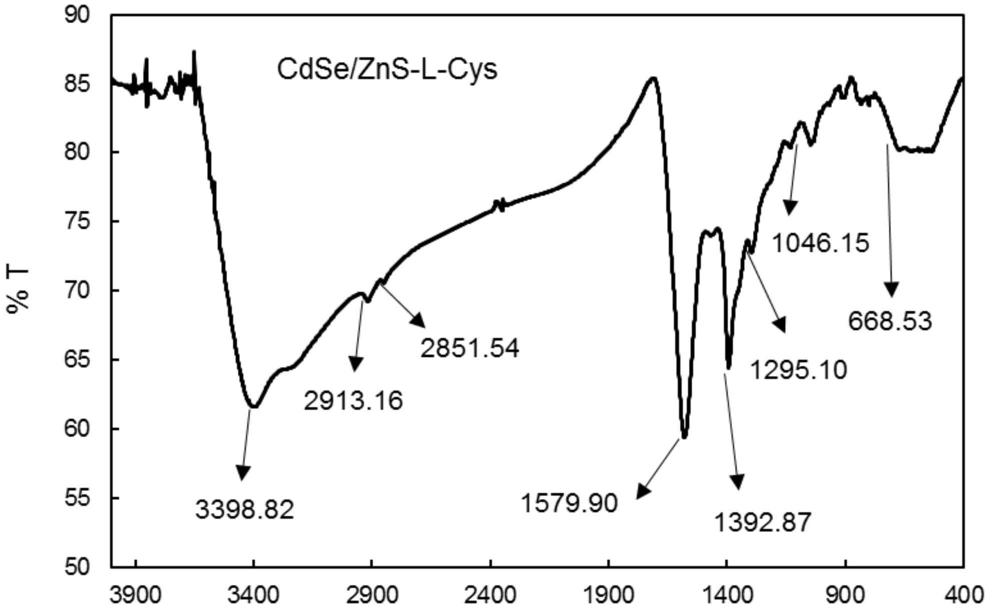


Figure 45: The FT-IR spectra of CdSe/ZnS-L-cys QDs

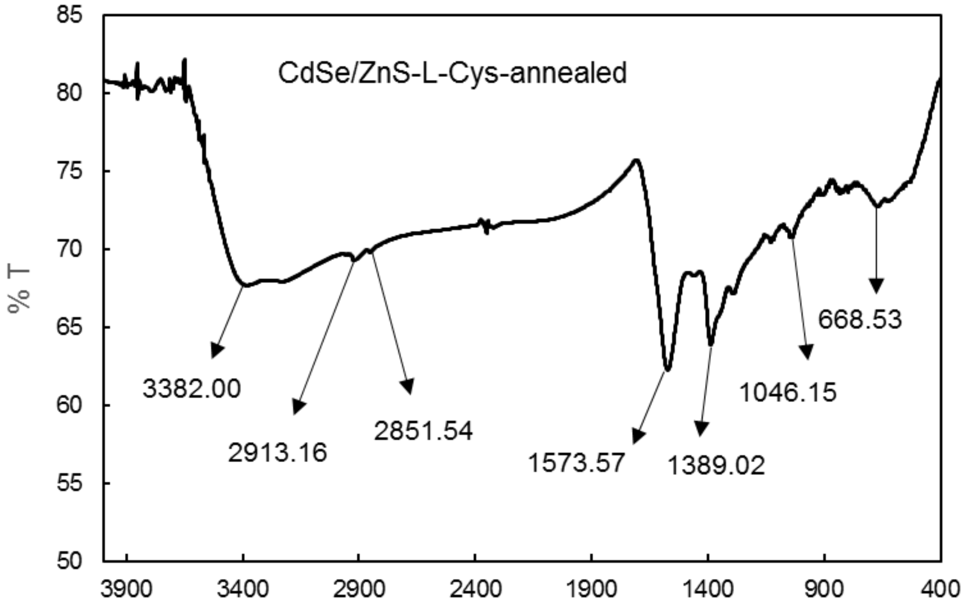


Figure 46: The FT-IR spectra of annealed CdSe/ZnS-L-cys QDs

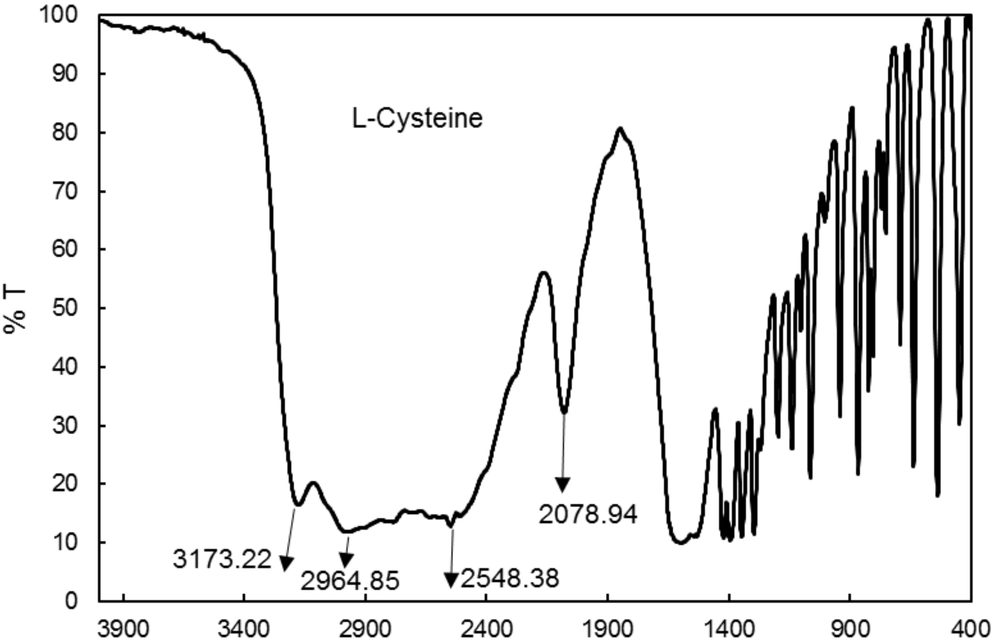


Figure 47: The FT-IR spectra of L-cys

CdSe/ZnS-NAC QDs

In order to compare the presence of NAC on the surface of the QDs, IR spectra of free NAC, NAC-capped CdSe and CdSe/ZnS are shown in Figure 50–Figure 50. Two absorptions at 1390.13 cm^{-1} and 1595.94 cm^{-1} for CdSe/ZnS-NAC QDs represent the asymmetric −COO− and symmetric −COO− vibrations in NAC, which indicates the deprotonation as a consequence of binding of NAC on the surface of the QDs.

The presence of stretching absorption of O-H can be seen at ~3422 cm^{-1}, and the molecules of NAC on the surface of QDs are associated with each other through intermolecular hydrogen bonds. Moreover, the absence of –SH group at band 2550–2750 cm^{-1} reveals the cleavage of S-H bonds and confirms the Cd-S bonds, which is due to the bonding of NAC to the surface of QDs.

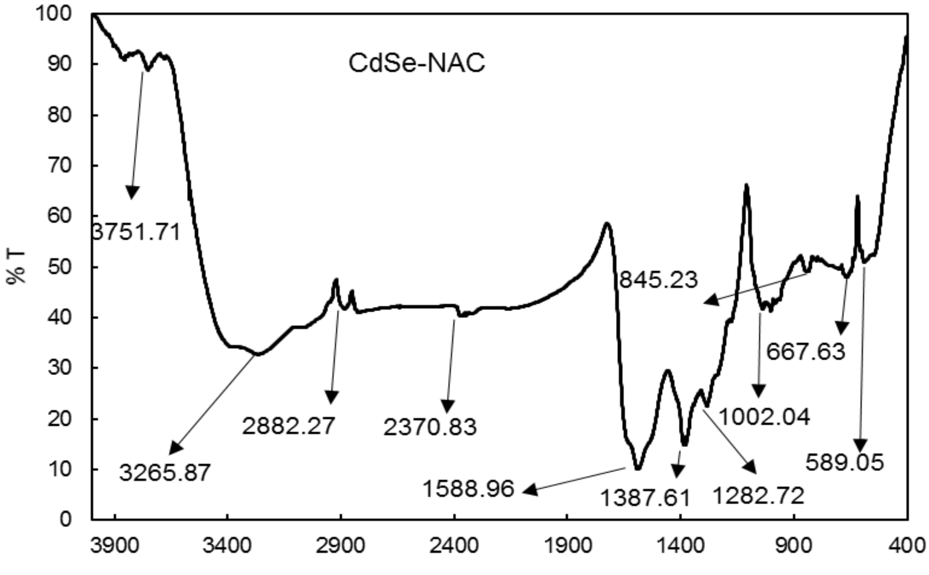


Figure 48: The FT-IR spectra of CdSe-NAC QDs

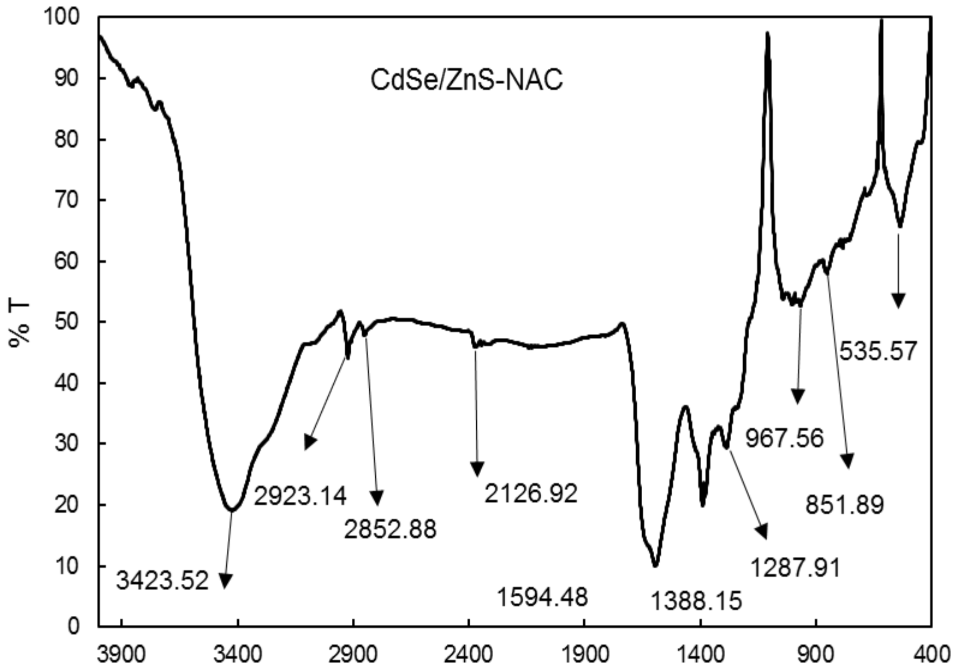


Figure 49: The FT-IR spectra of CdSe/ZnS-NAC QDs

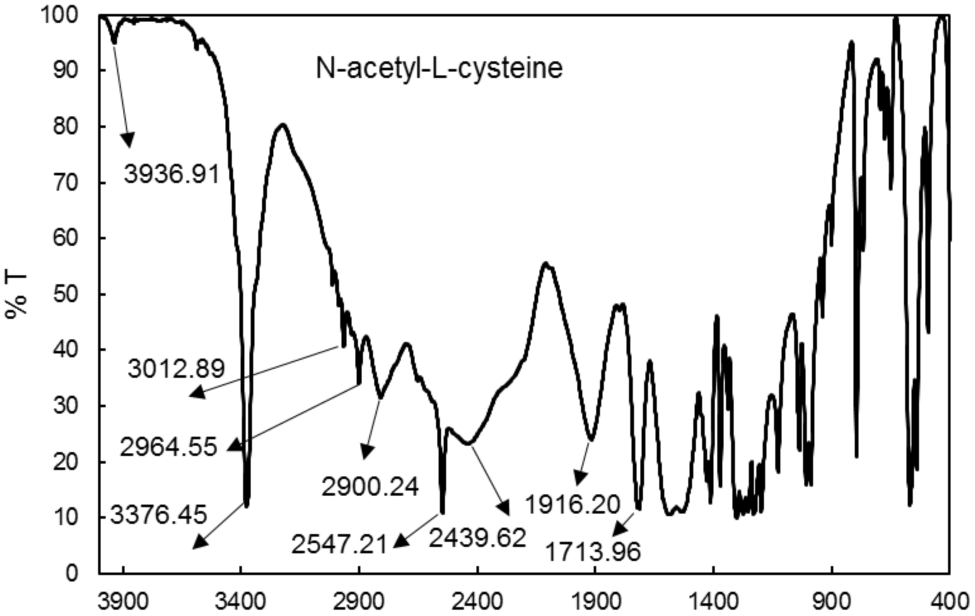


Figure 50: The FT-IR spectra of free NAC

CdSe/ZnS-GSH QDs

Confirmation of the attachment of GSH to the QDs was provided by FT-IR spectroscopy in Figure 53–Figure 53. From this figure, it is clear that the infrared spectra of the two QDs are similar. This indicates that GSH binds on the surfaces of the two QDs in the same way. The most characteristic peak at 2524 cm^{-1} in the spectrum of pure GSH related to the stretching vibration of the –SH group. The symmetric stretching vibration of the carboxylic group is at 1396 cm^{-1}. Moreover, the peak at 550 cm^{-1} pertains to the bending vibration of the –SH group. More importantly, the absence of the band of the

−SH group at 2550–2750 cm^{-1} confirms the Cd-S bond formation and successful attachment of GSH to the surface of QDs while the carboxyl group of GSH provides water solubility.

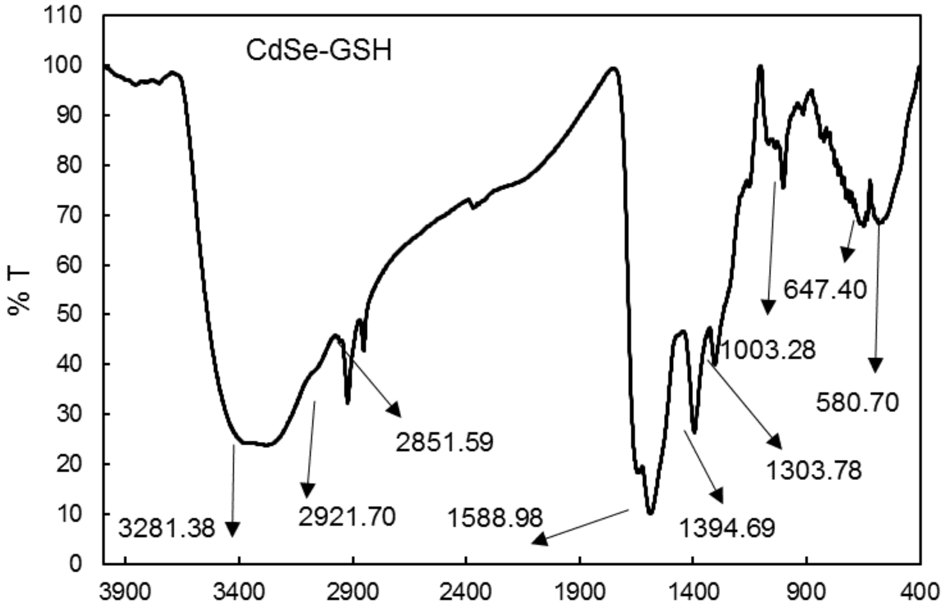


Figure 51: The FT-IR spectra of CdSe-GSH QDs

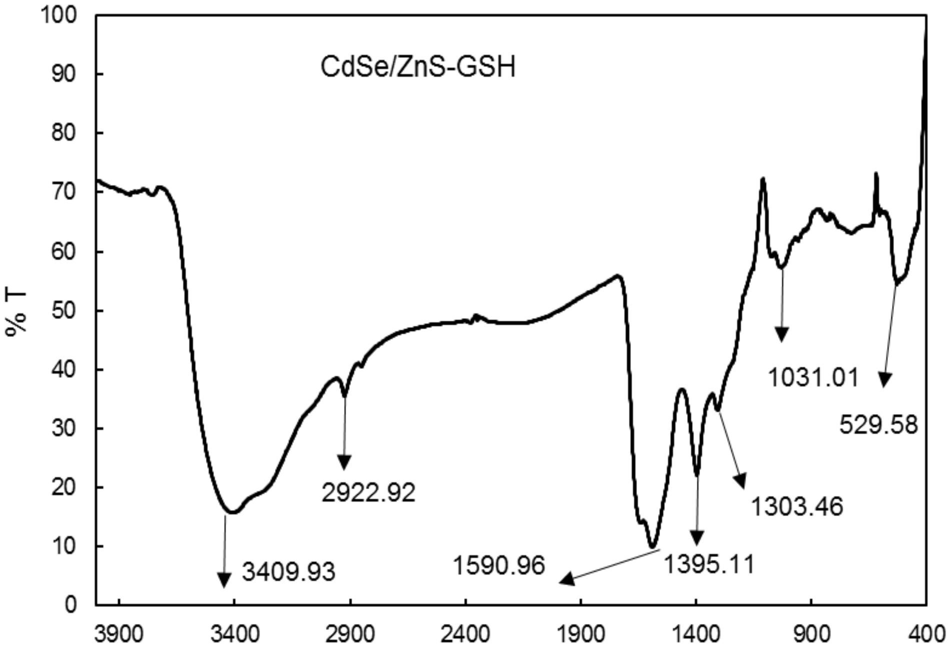


Figure 52: The FT-IR spectra of CdSe/ZnS-GSH QDs

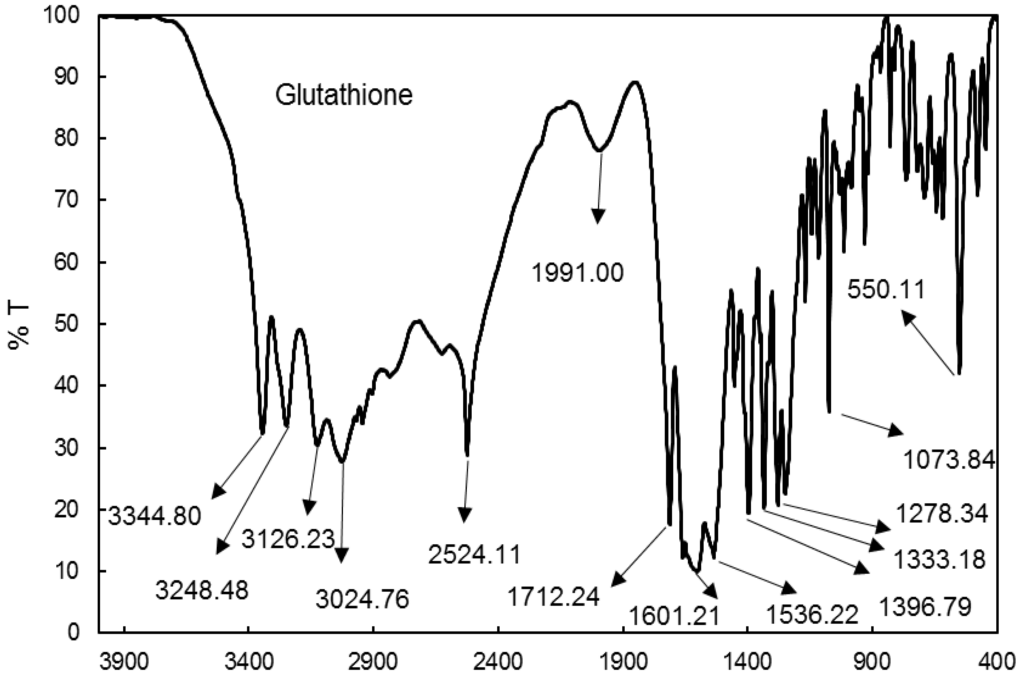


Figure 53: The FT-IR spectra of GSH

5.4.1.6 Stereo microscopy

The stereo- or stereoscopic microscope is an optical microscope that can be applied for low magnification observations of a sample, typically using light reflected from the surface of an object rather than transmitted through it (Nothnagle et al., n.d.). The direct observation of large CdSe/ZnS-L-cys QD crystals was performed under a stereomicroscope (Figure 54). The structure of L-cys-capped CdSe/ ZnS QD crystals were irregular and the surface was partially covered by QD powder. These crystals were ground to produce QD material for further study.

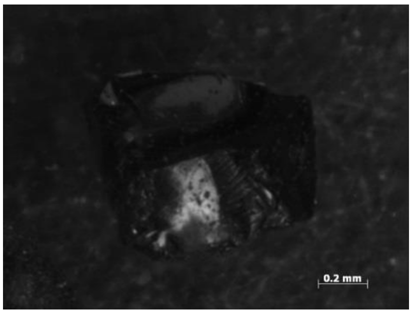


Figure 54: Stereo microscope image of bulk CdSe/ZnS-L-cys QD crystals

5.4.1.7 TEM analysis

TEM analysis was carried out to enable qualitative probing of the morphology and average particle size distribution of the QDs. As shown in Figure 55, it is clearly evident that the QDs were monodispersed, spherical in shape and dispersed well in aqueous solution. The morphological display of the QDs also shows that they were almost homogenous in nature. The estimated average particle size distribution of the CdSe-L-cys and CdSe/ZnS-L-cys QDs were 4.3 nm and 6.0 nm, while the average particle size for CdSe-GSH and CdSe/ZnS-GSH QDs were 5.2 nm and 6.2 nm. The size distribution was 4.0 nm and 6.0 nm for CdSe-NAC and CdSe/ZnS-NAC QDs, respectively.

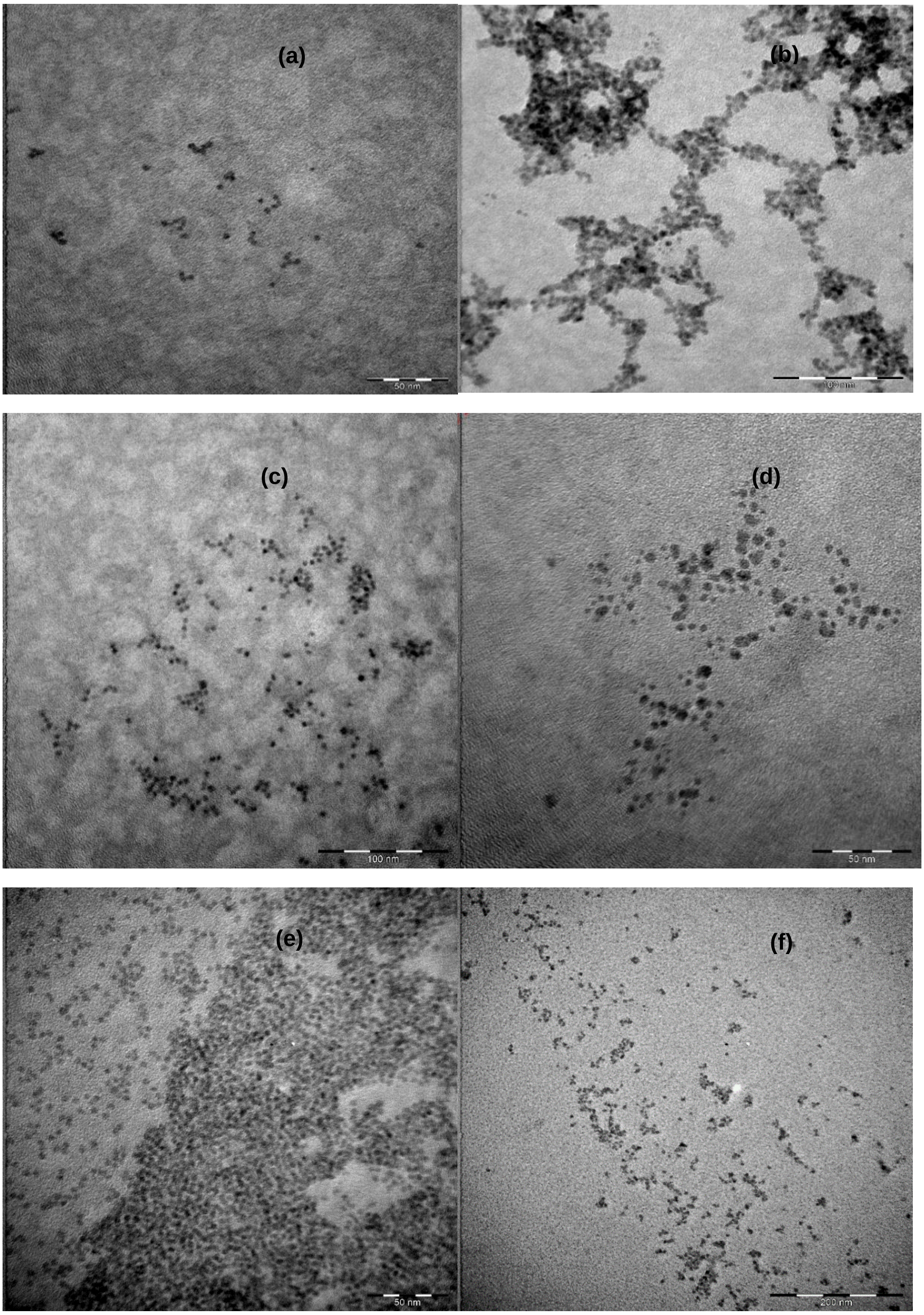


Figure 55: TEM images of CdSe-L-cys QDs (a), CdSe/ZnS-L-cys QDs (b), CdSe-GSH (c), CdSe/ZnS-GSH (d), CdSe-NAC (e) and CdSe/ZnS-NAC (f)

5.4.2 Graphene QDs

5.4.2.1 Infrared characterisation

Infrared analysis was done to confirm formation of GO from graphite. To get the GQDs into powder form, a small amount of the GQDs solution was freeze-died before infrared analysis. The results in Figure 56 show that GO was successfully made as the characteristic bands for GO were observed. These characteristic bands are: the carbonyl band (1723.07 cm^{-1}), the aromatic carbon band (1588.81 cm^{-1}), the carboxy band (1222.92 cm^{-1}), the epoxy band (1222.92 cm^{-1}) and the alkoxy band (1046.15 cm^{-1}). The broad band at around 3350 cm^{-1} is due to O-H stretching vibrations.

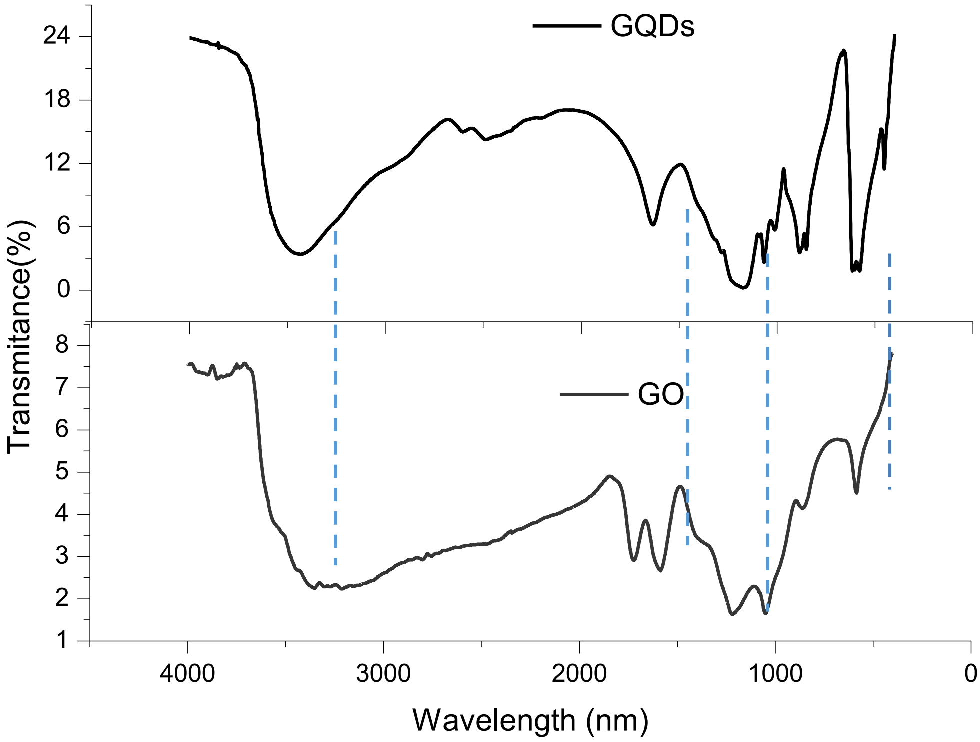


Figure 56: Infrared spectrum for GO and GQDs showing characteristic functional groups. These confirm successful oxidation of graphite starting material

5.4.2.2 HRSEM

HRSEM was used to study the morphology of the GO from exfoliation of graphite precursors. The HRSEM images show that the graphite had characteristic flat carbon sheets stacked into layers (Figure 57a) and after exfoliation to GO the sheets become folded into crumpled silk waves (Figure 57b). This transformation is due to the harsh oxidation conditions that the graphite was subjected to.

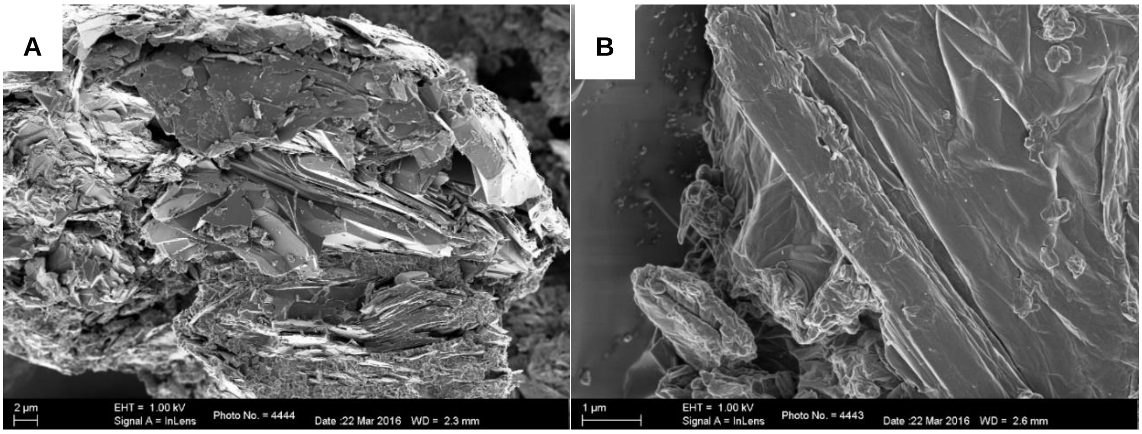


Figure 57: HRSEM image of (a) graphite powder; (b) GO from exfoliation

5.4.2.3 XRD of GO

Powder XRD was used to study the crystal phase nature of both graphite precursor and the GO. A clear distinction was observed between the two (Figure 58). Graphite had an intense peak at 2θ = 30.6 degrees and after converting to GO, the peak diminished. GO had a week characteristic peak at 2θ = 10 degrees, which indicates a loose layer-like structure. The interlayer spacing of this peak depends on water layers in the gallery space of the materials as well as synthesis method (Liu et al., 2002).

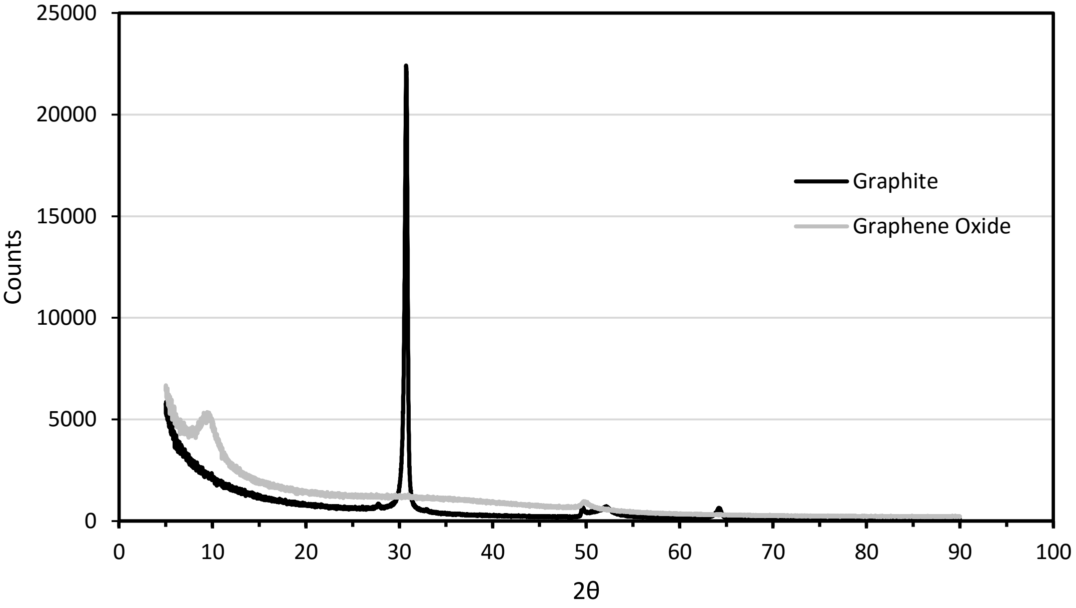


Figure 58: XRD pattern for graphite and GO synthesised using modified Hummer's method

5.4.2.4 Raman analysis

The Raman spectra for the graphite precursor and GO are shown in Figure 59. The characteristic *D* (1360 cm^{-1}) and G (1600 cm^{-1}) bands were observed for both materials but were more pronounced for GO. This confirmed successful exfoliation of the graphite precursor material. The other spikes in the spectra are due to instrument noise. The *D* band corresponds to the plane termination of disordered graphite-containing vibrating carbon atoms while the *G* band corresponds to vibrating sp^2 bonded carbon atoms (Wei et al., 2013).

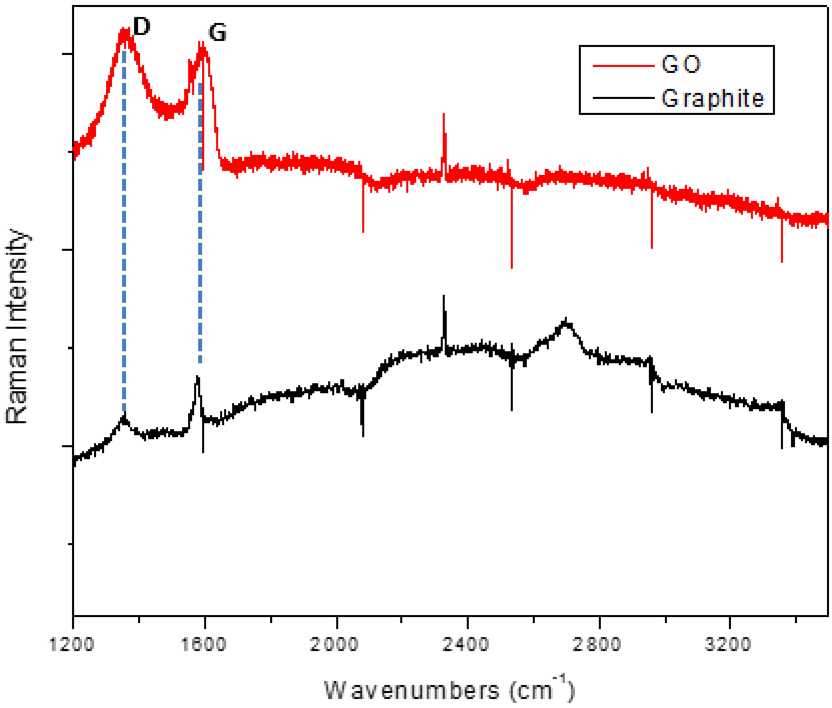


Figure 59: Raman spectra of graphite powder and GO

5.4.2.5 Optical Properties

Figure 60 shows UV-vis absorption spectra of GO and the prepared GQDs, which were all dispersed in water. The GQDs were found to have a strong UV absorption peak at 298 nm, which is attributed to π→π* transition of aromatic sp^2 domains in the graphene structure, and a shoulder around 340 nm, which is ascribed to n→π* transition of C=O bonds or order connected groups on the GQD surfaces (Zhu et al., 2015). The position and intensities of these characteristic peaks have been shown by Zhang et al. (2016) to be dependent on the size distribution of the GQDs, which itself is dependent on the synthetic route.

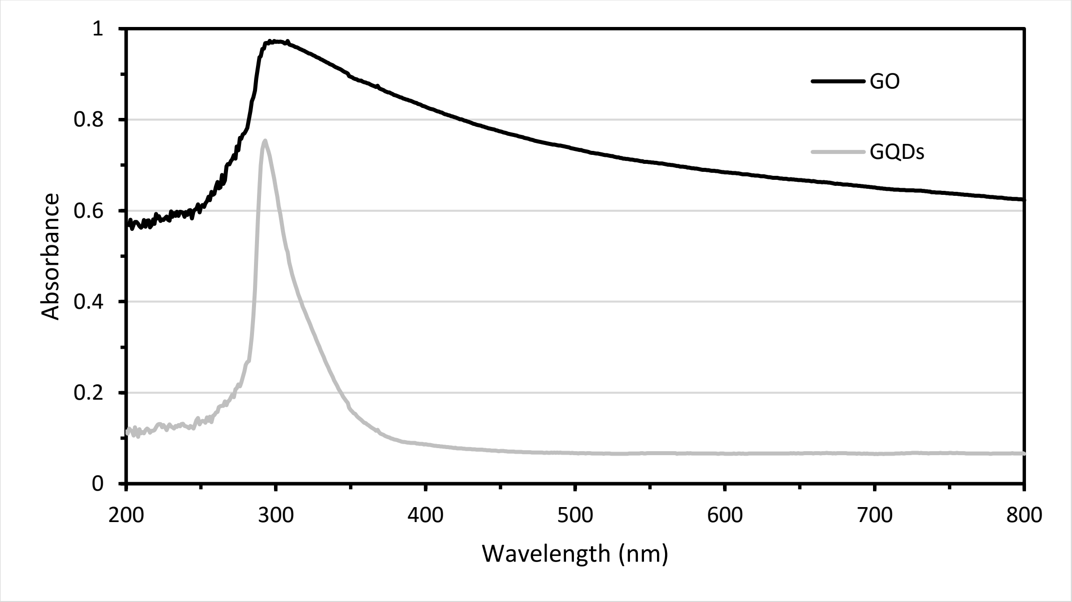


Figure 60: UV-vis spectrum of GO and of GQDs

Figure 61 shows fluorescence emission spectra of the GQDs. The intensity of emission peaks was observed to be dependent on the excitation wavelength and had a red-shift as the wavelength was changed from 340 nm to 500 nm. This behaviour is common with fluorescent carbon materials and in the case of GQDs it may be due to the presence of differently sized dots, surface chemistry, or emissive traps. Another striking feature is that the peaks are much wider than that of semiconductor QDs. This may be due to inhomogeneous chemical structure and different emission centres (Zhu et al., 2015). Based on the results shown in Figure 61, 340 nm was chosen as the optimum excitation wavelength for subsequent sensing studies.

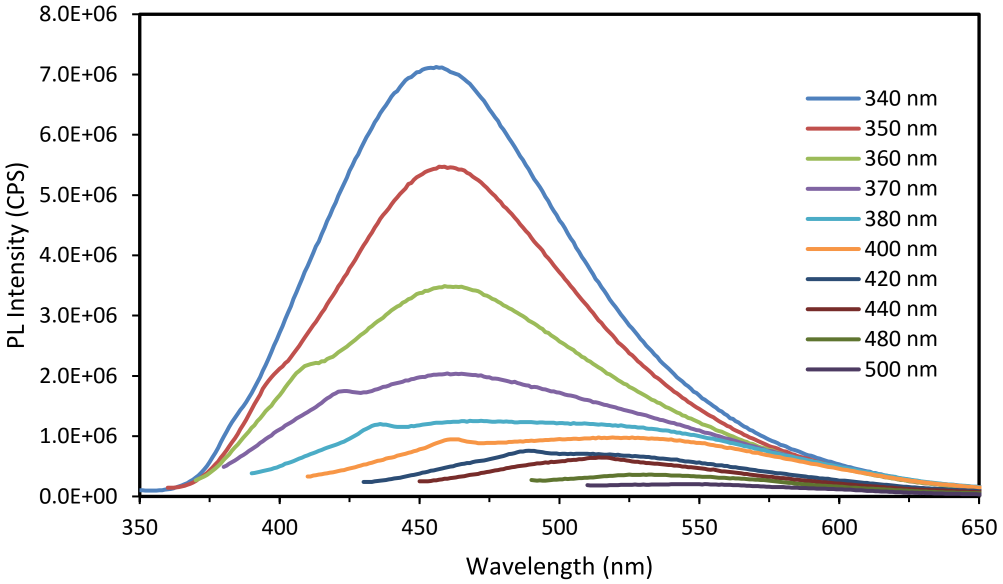


Figure 61: Fluorescence spectra of GQDs at different excitation wavelengths showing a decrease in PL intensity with increasing excitation wavelength

5.5 Conclusion

Water-soluble semiconductor CdSe/ZnS QDs capped with L-cys and NAC were synthesised by a one-pot process as novel fluorescence sensor for the potential determination of triclosan and acetamino-phen in water samples. Their structures were characterised by UV/Vis spectroscopy, fluorescence spectroscopy, FT-IR and powder XRD. The higher band-gap ZnS was directly grown onto monodisperse CdSe QD cores to protect the surface from the leakage of toxic cadmium ions into the solution in use. Furthermore, thiol ligands (L-cys and NAC) that were attached to the surface of ZnS not only provided water solubility but also may further prevent the leaching of cadmium ions. These QDs exhibited a remarkable stability which is attributed to the thiolate group anchoring to the surface of nanoparticles.

GQDs have been successfully prepared from oxidation of graphite as starting material. The GQDs had excellent optical properties, with high fluorescence intensity peaks depending on the excitation wavelength. TEM analysis still has to be done to understand the size distribution of the GQDs.

A preliminary study was carried out to investigate their application as sensors for PAHs in water, since graphene materials have been previously shown by our group to have strong affinity for PAHs because of π-π interaction. Upon interaction with the sensor, the PAHs were found to have a quenching effect on the fluorescence intensity of the GQDs, due to energy transfer from the GQDs to the PAHs, which could be through a FRET mechanism. The sensor showed good sensitivity towards phenanthrene and additional PAHs are to be investigated, namely, anthracene, pyrene and naphthalene. In addition, investigation of the effects of potential interferences by other aromatic compounds on the sensor is to be done.

6 TESTING OF FLUORESCENCE SENSOR MATERIALS USING STANDARD SOLUTIONS AND THE DEVELOPMENT OF THE FLUORESCENCE SENSOR PROTOTYPES

By Hanieh Montaseri[11], Sifiso Nsibande[10], Oluwasesan Adegoke[12] and Patricia Forbes[10]

6.1 Introduction

6.1.1 Introduction to the fluorescence sensing of acetaminophen and triclosan

Several analytical techniques have been reported to determine acetaminophen and triclosan in different matrices including HPLC (Dinç et al., 2014; Shen et al., 2012b), electrochemical analysis (Gorla et al., 2016; Yola et al., 2015), capillary zone electrophoresis (Wang et al., 2013a; Zhao et al., 2006), spectrophotometry (Khaskheli et al., 2007; Kysliak & Smyk, 2010), and chemiluminescence (Easwaramoorthy et al., 2001; Song et al., 2007), but these methods usually have disadvantages in terms of analytical running costs, complexity, environmentally unfriendly solvent use, expensive instrumentation, long analysis time, as well as the need for highly skilled technicians and laborious sample pretreatment (Guo et al., 2009; Goyal et al., 2010). Rapid and sensitive screening methods including fluorescence methods may provide desirable alternatives, because of their simplicity, fast experimental process, low cost and high sensitivity towards different molecular structures based on different fluorescence emission wavelengths (Li et al., 2009a). Further, fluorescence methods provide additional advantages including high sensitivity, simple sample treatment requirements and rapid response times (Yuan et al., 2013). QDs as optical probes have been used extensively since their first application (Bruchez et al., 1998; Chan & Nie, 1998).

Interaction of analytes with QDs, which takes place at the surface of the QDs, affects the efficiency of the radiative recombination inducing PL activation or quenching (Frasco & Chaniotakis, 2010). Generally, fluorescence quenching includes two types of quenching, namely, dynamic quenching and static quenching. Fluorescence lifetime measurements, temperature-varying experiments and absorption spectrum measurements are often used to identify the kind of quenching and its mechanism (Cheng et al., 2006).

The PL “turn-on” mode is preferable to the PL quenching mode as a variety of factors other than analytes can induce the ultimate PL “off” state. The PL “off-on” conversion in the “turn-on” mode is easier to detect and can reduce the chance of false positives and is more amenable to multiplexing, i.e. the simultaneous use of several detectors that uniquely respond to various analytes (Xu et al., 2011).

When the target analyte and coexisting compounds show a similar luminescence response, sensing and recognising the analyte are challenging. In order to improve the selectivity of QDs, MIPs may be employed (Xu & Lu, 2015). The synthesis of MIPs is based on the copolymerization of functional monomers and cross-linkers in the presence of analyte as a template (Wang et al., 2014). The template is then removed by an appropriate solvent and recognition sites complementary in size, shape and functionality to the template remain. Additionally, MIPs have generated great attention due to their reusability, physical robustness, high selectivity, thermal stability, easy preparation and effective cost (Huy et al., 2014).

Here we focus on the application of the CdSe/ZnS QDs with different capping agents, which we have synthesised and characterised as detailed in Chapter 5 of this report, in the fluorescence sensing of acetaminophen and triclosan in water.

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6.1.2 Introduction to pesticide sensing

Atrazine is used extensively in agriculture for controlling weeds and it may eventually contaminate water systems through leaching, run-off and spray drift. It has been reported to occur in South African water systems by several studies (Dabrowski, 2015a, 2015b; Dabrowski & Balderacchi, 2013; Dabrowski & Schulz, 2003; Dabrowski et al., 2013; Dabrowski et al., 2014; Du Preez et al., 2005; Patterton, 2013). This makes atrazine a priority ECP that warrants the development of sensitive and economical analytical methods, which can be used routinely as an alternative to current conventional methods, which are relatively expensive.

Here we report on our current work regarding the development of a QD fluorescence sensor for atrazine detection in aqueous solutions.

Atrazine can interact with L-cys ligands around the QDs through hydrogen bonding with the carboxylic groups of the L-cys molecule (Welhouse & Bleam, 1993) as illustrated in Figure 62. Upon irradiation, energy can then be transferred between the atrazine QD and the target molecule (i.e. atrazine) via different mechanisms such as FRET or electron energy transfer. This interaction may result in enhancement of the fluorescence signal of the QDs, thus a “turn-on” sensor can be designed based on this interaction. Different L-cys-capped QDs were investigated as possible probes for atrazine detection. These were CdSe, ZnSeS/ZnTe/ZnS and CdTeSe/ZnSe/ZnS.

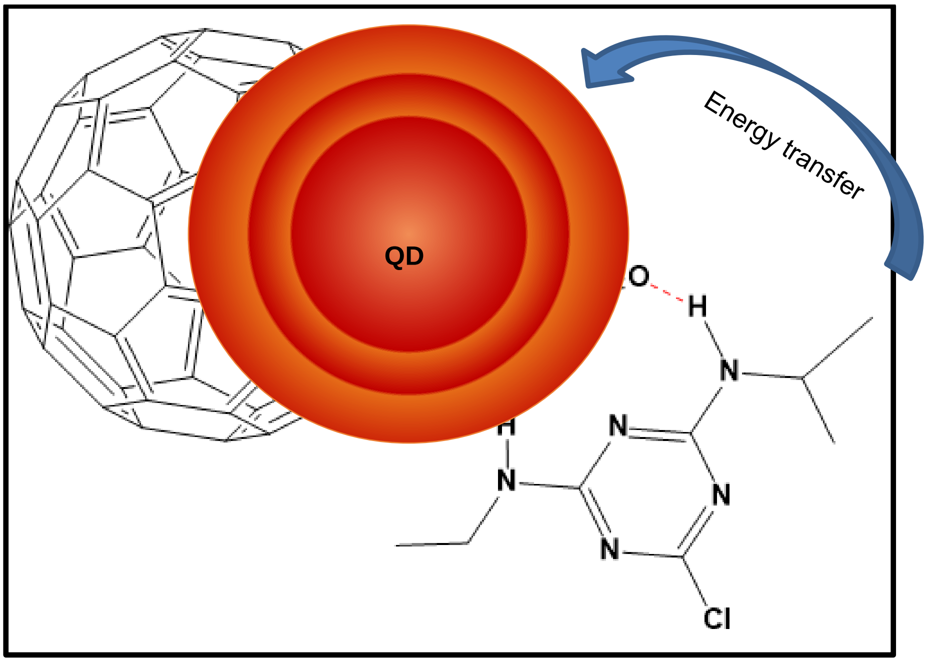


Figure 62: Schematic diagram showing the postulated hydrogen bonding interaction (dashed red lines) between atrazine and L-cys ligands around QD nanoparticles. The energy transfer between the QD and atrazine results in fluorescence enhancement

6.1.3 Introduction to PAHs sensing with graphene QDs

PAHs are potentially harmful pollutants emitted into the environment from a range of sources as a consequence of combustion processes (Chimuka et al., 2015). These compounds can occur in natural waters through point and non-point source discharges to surface water, groundwater and drinking water (Manoli & Samara, 1999). There are serious concerns around exposure to PAHs as some of them have been acknowledged as probable human carcinogens (Kim et al., 2013b), hence there is need for continuous environmental monitoring of these compounds. In the previous chapters of this report the importance of monitoring these compounds in water systems, especially in South Africa has been discussed.

In order to develop a fluorescence sensor for this application, a synthetic route to couple L-cys-CdSeTe/ZnSe/ZnS QDs to graphene was successfully completed to form a stable novel nanocomposite. The material was characterised and was found to be a suitable sensor for PAHs in water. An LOD of 0.19 $\mu g \cdot L^{-1}$ was obtained for phenanthrene under optimum conditions, while the LODs of anthracene, pyrene and naphthalene were estimated to be ~0.26 $\mu g \cdot L^{-1}$. These results have been published in *Talanta* (Adegoke et al., 2015), wherein a full description of the methodology can be found.

6.1.4 Introduction to QD immobilisation

The development of luminescence-based sensing devices ideally requires immobilisation of the sensing indicators, the QDs, onto or into solid supports to form active solids for working in flowing solutions and allowing for reuse. Forming such active materials also brings the advantage of reducing the potential for leaching of the nanocrystals into the solution while equilibrium with the target analyte is being established (Jorge et al., 2007). During the immobilisation process, however, the luminescence efficiencies of the QDs have been reported to be significantly reduced due to aggregation and oxidation of the QDs and poor adhesion on the support material (Yoo et al., 2015). The challenge therefore, is to retain the properties of the QDs after the immobilisation process.

Several methods have been reported for QD immobilisation on solid supports for different applications. Different silicon based materials have been used to immobilise QDs for different applications. These include silica beads (Lee et al., 2012; Wang et al., 2009b; Yoo et al., 2015) sol-gel matrices (Algar & Krull, 2011; Reisfeld & Saraidarov, 2006; Sotelo-Gonzalez et al., 2012) and polydimethylsiloxane (PDMS) (Bodas & Khan-Malek, 2007; Botros & Savage, 2012; Khadka, 2013; Moore, 2013; Tao et al., 2013).

In this report, we focus on PDMS because of its advantages, which include low cost, no toxicity, flexibility, as well as its ability to absorb organic non-polar analytes. We therefore sought to fabricate thin films of PDMS embedded with QDs and to study the properties of the resulting PDMS-QD material with the intention to use it to detect ECP compounds in future work.

6.2 Experimental

6.2.1 Materials and methods for acetaminophen sensing: Determination with L-cys-capped CdSe/ZnS QDs

Acetaminophen, GSH, and L-cys were purchased from Sigma-Aldrich. Methanol HPLC grade and triclosan were purchased from Merck. An ultrapure Milli-Q Water System was used for sample preparation. QDs were prepared as previously detailed.

Foreign analytes including dopamine hydrochloride (DA), ascorbic acid (AA), uric acid (UA), epinephrine hydrochloride (EP), 4-aminophenol (AP), L-tryptophan (TRY), ketoprofen (KTP), sulfamethoxazole (SM), oestradiol (ES), L-Dopa, and diclofenac sodium salt (Dic) as foreign analytes were also purchased from Sigma-Aldrich.

All measurements were performed under the same conditions: the excitation and emission slit width was 5 nm. The fluorescence intensity was measured at an excitation wavelength of 300 nm. A typical procedure for the detection of acetaminophen is described as follows: 1.0 mg of CdSe/ZnS-L-cys was dissolved in 3 mL Millipore water and a standard solution containing different amounts of acetaminophen was added. The corresponding PL spectra were recorded after different periods of time. Acetaminophen standard solutions were prepared within the range of 3.0×10^{-9}–1.0×10^{-7} $mol \cdot L^{-1}$ by dissolution in H_2O.

Sensing was carried out by placing 2 mL of QDs solution in a quartz cuvette followed by addition of 500 µL of acetaminophen solution standard solution. It took about 5 minutes for the stabilisation of the PL profile and this PL intensity was set as F. Moreover, the PL intensity of QDs in the presence of

500 µL of water was set as F_0. Through the variation of acetaminophen concentration, a series of F values were obtained.

6.2.2 Materials and methods for triclosan sensing: Determination with GSH-capped CdSe/ZnS QDs

GSH was purchased from Sigma-Aldrich. Methanol HPLC grade and triclosan were purchased from Merck. An ultrapure Milli-Q Water System was used for sample preparation. QDs were prepared as previously detailed.

1.5 mg of CdSe/ZnS-L-cys was dissolved in 3.0 mL of Millipore water. Triclosan standard solutions were also prepared within the 1×10^{-8}–5×10^{-8} mol·L^{-1} concentration range by dissolution in H_2O/MeOH (2:1) mixture. Sensing was carried out by placing 2 mL of QDs solution in a quartz cuvette followed by adding 200 µL of triclosan standard solution. The fluorescence intensity was measured at different time intervals at excitation wavelength of 300 nm with excitation and emission slit width of 5 nm.

6.2.3 Materials and methods for pesticide sensing

For sensing experiments, a small amount of QDs (~1 mg) was dissolved in about 15 mL of Millipore water and thoroughly shaken to get a homogenous solution, which was used for sensing. Atrazine standard (98.8% purity) was purchased Sigma-Aldrich while ethanol (EtOH) 99.5% purity was bought from Merck (Pty) Ltd.

Atrazine standard solutions were prepared in H_2O/EtOH (2:1) mixture and the concentration range was 1–5 $\times 10^{-7}$ mol·L^{-1}. For each sensing experiment, fluorescence measurements of 1 mL of the QD solution were taken followed by spiking with 100 µL of analyte solution. An equilibration time of 5 minutes was allowed before taking fluorescence measurements of the spiked QD solution. The excitation wavelengths were 490, 470, and 350 nm for CdSe, CdTeSe/ZnSe/ZnS and ZnSeS/ZnTe/ZnS QDs respectively. All PL measurements were done with a Horiba Jobin Yvon FluoroMax-4 spectro-fluorometer.

6.2.4 Materials and methods for PAHs sensing with GQDs

Four PAH compounds (anthracene, naphthalene, pyrene and phenanthrene) were selected to test the sensitivity of the GQD probe towards PAHs. These PAHs are of environmental and human health concern, and have been listed as priority PAHs by the US EPA (2010). Moreover, they have been reported to occur in water systems due to their low molecular weight (Chimuka et al., 2015; Qin et al., 2014; Zhou & Maskaoui, 2003).

PAH standards were purchased from Sigma-Aldrich, ethanol (EtOH) was from Merck (Pty) Ltd, and Millipore water that was used for all experiments was from a Milli-Q system. PAH standard solutions were prepared within the 1–5 $\times 10^{-7}$ mol·L^{-1} concentration range by dissolving the solids in H_2O/EtOH (2:1) mixture. To improve their solubility, they were first dissolved in small amounts of toluene. Sensing was carried out by placing 3 mL of GQDs solution in a quartz cuvette followed by adding 200 µL of analyte PAH standard solution. For all the sensing measurements the GQDs and PAHs were allowed an equilibration time of exactly 5 minutes before taking fluorescence measurements to ensure interaction of the analytes with the sensor.

6.2.5 Preparation of QD-PDMS films

Hydrophobic CdSeTeS QDs were prepared as reported (Adegoke et al., 2015). The TOPO ligands on the surface of these QDs allows for dispersion in the PDMS matrix. About 1 g of QD powder was dissolved in chloroform and a few drops of this solution were added to about 4 g of PDMS pre-polymer (base). This was followed by thorough mixing and sonication to disperse the QDs in the PDMS matrix. The resultant mixture was then degassed in a desiccator with vacuum. About 0.5 mL (~0.5 g) of PDMS curing agent was added followed by thorough stirring and degassing. The solution was spin-coated onto glass slides at 300 rpm for 1 minute. This resulted in spreading of the QD-PDMS on the glass slides to form a thin layer. The slides were then placed in the oven at ~90°C and were baked for about 15 min after which they were removed from the oven and cooled to room temperature for fluorescence analysis. Spin-coating was done using a Laurell spin coater (Model: WS-650MZ-23NPPB).

6.3 Results and Discussion

6.3.1 Acetaminophen sensing

6.3.1.1 Fluorescence properties of acetaminophen standard solution

As acetaminophen itself fluoresces, we recorded its PL from 290 nm to 520 nm as excitation wavelength in order to investigate the possible effect on the PL spectra of CdSe/ZnS-L-cys QDs. Figure 63(a) shows that the highest intensity was around the emission wavelength of 400 nm at the excitation wavelength of 300 nm.

In theory, FRET is a non-radiative process and if the emission spectrum of the donor overlaps with the absorption spectra of the acceptor, energy transfer from the donor to the acceptor could occur through long range dipole-dipole interaction, leading to enhanced or quenched QD fluorescence signals. The rate of energy transfer depends on the relative orientation of the transition dipoles, spectral overlap and most importantly the distance between donor and acceptor (Duong et al., 2011).

In this work, the absorption spectra of CdSe/ZnS-L-cys QDs at 450-560 nm overlaps with the emission spectra of acetaminophen at 340-560 nm (Figure 63). Hence, QDs can absorb the emission energy from acetaminophen leading to the enhancement of QD fluorescence signals.

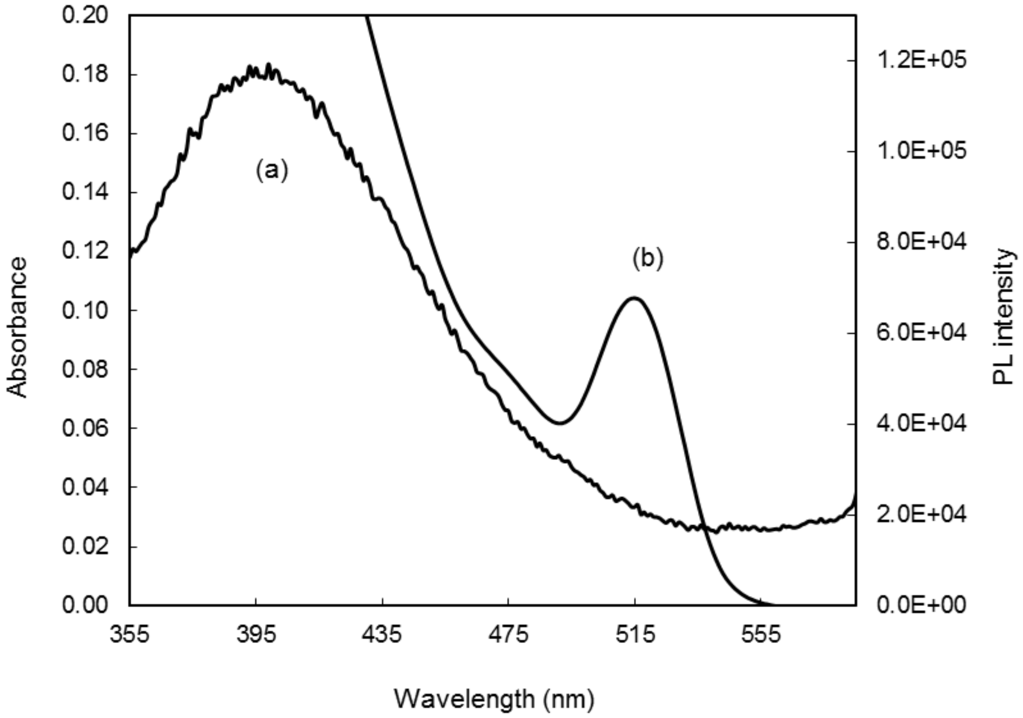


Figure 63: Florescence spectra of acetaminophen (1.0 × 10^{-5} mol·L^{-1}) at excitation wavelength 300 nm (a) and UV-Vis absorption spectra of CdSe/ZnS-L-cys QDs (b)

6.3.1.2 Effect of aqueous CdSe/ZnS-L-cys QDs concentration

Considering that concentration QD concentrations can influence the fluorescence intensity, different concentrations of CdSe/ZnS-L-cys (1.0 mg, 1.5 mg, 2.0 mg, 2.5 mg, 3.0 mg in 3.0 mL water) were prepared and 5.0×10^{-9} mol·L^{-1} of acetaminophen was added to each. It was found that high concentrations of aqueous CdSe/ZnS-L-cys decreased the sensitivity of the system and self-quenching of QDs occurred whereas, very low concentrations of QDs led to a narrowing of the linear range (Figure 64), therefore the optimum concentration was 1.0 mg in 3.0 mL water.

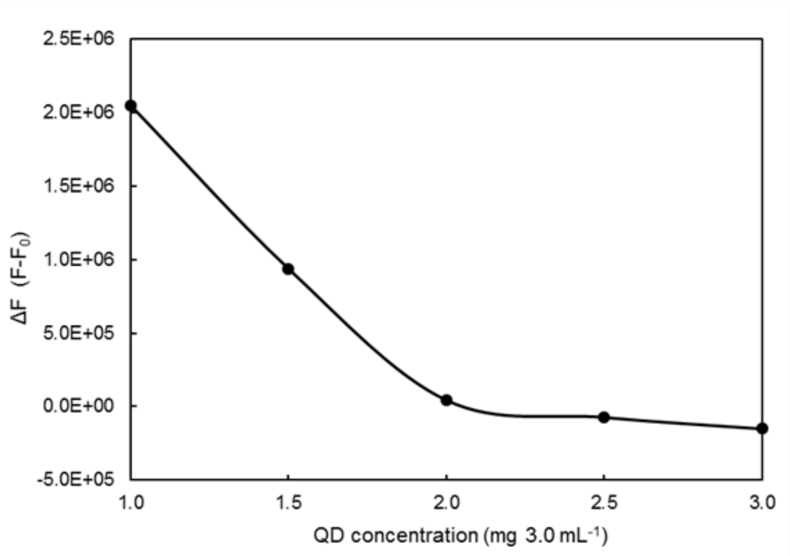


Figure 64: Effect of the concentration of aqueous CdSe/ZnS-L-cys QDs in the presence of 5.0×10^{-9} mol·L^{-1} acetaminophen, fluorescence intensity without acetaminophen (F_0), fluorescence intensity with acetaminophen (F)

6.3.1.3 Effect of incubation time

In order to investigate the reaction time of CdSe/ZnS-L-cys with acetaminophen, the fluorescence intensity of the system was tested at different time intervals in the presence of 5.0×10^{-9} mol·L^{-1} acetaminophen (Figure 65). The maximum fluorescence intensity was obtained after 5 min, indicating the reaction was completed within 5 minutes, which was thus used in subsequent experiments. A quenching effect of the PL intensity of the QDs was observed after 10 min.

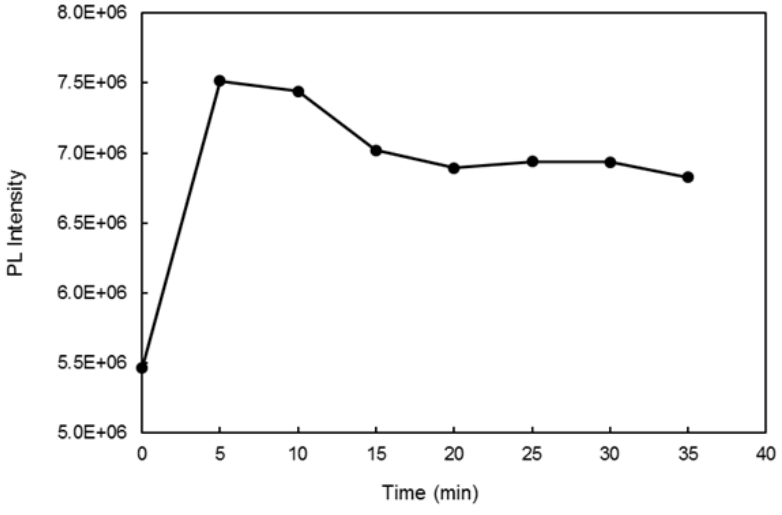


Figure 65: Effect of reaction time on the fluorescence intensity of L-cys-capped CdSe/ZnS QD-acetaminophen system

6.3.1.4 *Fluorescence behaviour of L-cys-capped CdSe/ZnS QDs in the presence of acetaminophen*

Under the optimal conditions as described above, the introduction of acetaminophen to the CdSe/ZnS-L-cys QDs resulted in an increment luminescence enhancement (Figure 66). Signal enhancement could be due to FRET from analyte (donor) to QDs (acceptor), which occurs when the emission band of the donor overlaps with the excitation band of the acceptor. The emission of the acceptor enhances and simultaneously the luminescence of the donor quenches (Goldman et al., 2005; Medintz et al., 2003). Here the fluorescence of the donor (acetaminophen) was of very weak intensity compared to that of the acceptor (QDs), therefore that of acetaminophen (as well as the fluorescence quenching thereof) is not visible in Figure 66.

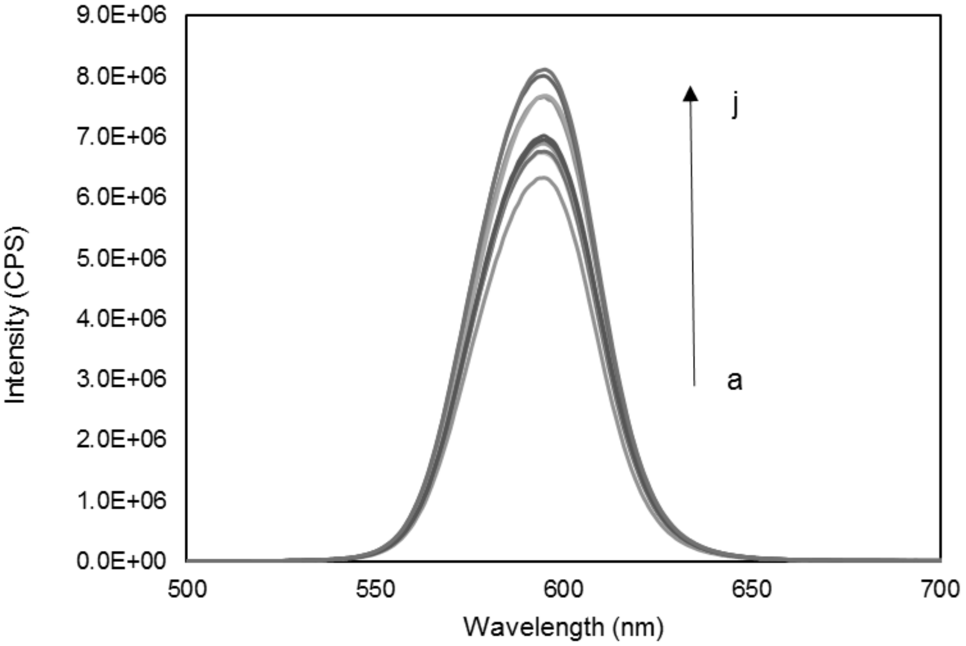


Figure 66: The fluorescence spectra of L-cys-CdSe/ZnS QDs in different concentrations of acetaminophen (mol·L^{-1}): (a) 0, (b) 3.0×10^{-9}, (c) 5.0×10^{-9}, (d) 7.0×10^{-9}, (e) 9.0×10^{-9}, (f) 3.0×10^{-8}, (g) 5.0×10^{-8}, (h) 7.0×10^{-8}, (i) 9.0×10^{-8}, (j) 1.0×10^{-7}

6.3.1.5 *Calibration curve, LOD and LOQ*

A plot of fluorescence intensity ($F\text{-}F_0$) versus acetaminophen concentration is shown in Figure 67, where F_0 and F are the fluorescence intensities of the QDs without and with different acetaminophen concentration respectively. A good linear relationship between $F\text{-}F_0$ and [acetaminophen] was observed from 3.0×10^{-9}–1.0×10^{-7} mol·L^{-1} ($r^2 = 0.98$).

The LOD and LOQ are defined by 3δ/m and 10δ/m, where δ is the standard deviation of blank measurement (n = 10) and m is the slope of the calibration curve. LOD and LOQ were found to be 4.22×10^{-9} mol·L^{-1} (or 4.22 nmol·L^{-1}) and 1.44×10^{-8} mol·L^{-1} (or 14.4 nmol·L^{-1}) respectively. The data revealed that the proposed method is suitable for the determination of acetaminophen.

Acetaminophen has been found at levels 7.0–10 000 ng·L^{-1} (4.6×10^{-11}–6.6×10^{-8} mol·L^{-1}) in surface water (Daughton & Ternes, 1999). A multitude of techniques have been developed for the determination of acetaminophen, including one semiconductor QD fluorescence-based method with the linear range and detection limit of 1.0×10^{-8}–1.6×10^{-7} and 4.2×10^{-9} mol·L^{-1} respectively (Li et al., 2012a). This shows the potential of our sensor for detecting the low concentrations of acetaminophen typically found in water samples and that it is fit for purpose.

In comparison, traditional methods such as HPLC-ESI-MS/MS provide detection limits of ~1.3×10^{-8} mol·L^{-1} in surface water (Roberts & Thomas, 2006); therefore, the LOD obtained with the CdSe/ZnS-L-cys probe for acetaminophen has competitive sensitivity and also provides an analysis at a much lower cost (refer to Cost Benefit Analysis section of this report).

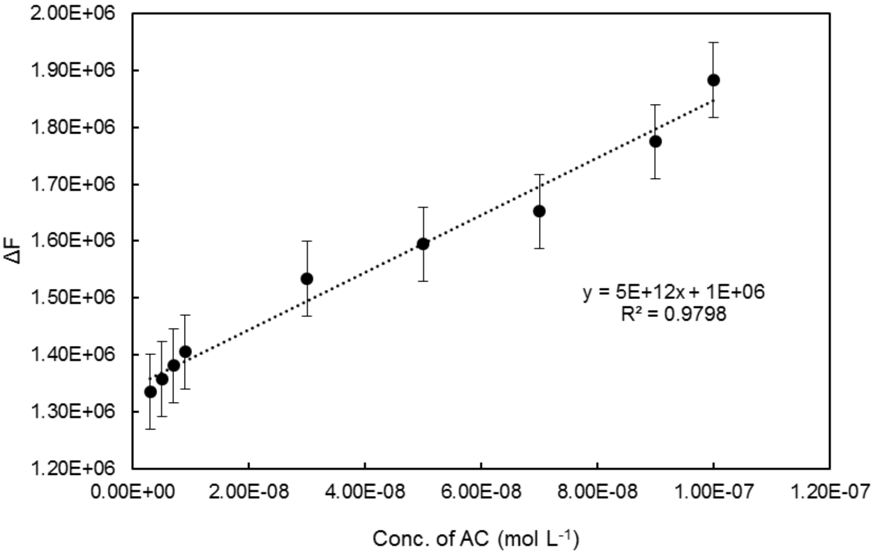


Figure 67: Linear graph of ΔF versus acetaminophen concentration based on detection using L-cys CdSe/ZnS QDs

6.3.1.6 Effect of other potentially interfering analytes on CdSe/ZnS-L-cys QDs

The possible effect of other analytes on the QD fluorescence was investigated under optimum conditions in the presence of 1.0×10^{-7} mol·L^{-1} of each analyte after 5 min equilibration time. In the presence of other pharmaceutical analytes, the PL intensity of CdSe/ZnS-L-cys QDs was enhanced (Figure 68), which indicates that the prototype QD probe may be employed for the detection of this class of ECP analyses. The enhancement effect followed the order:

Acetaminophen~KTP>TRY>SM>EP>DA>AA>AP>Dic>ES>UA>L-Dopa. Therefore, acetaminophen was chosen for further PL testing using the CdSe/ZnS-L-cys QDs.

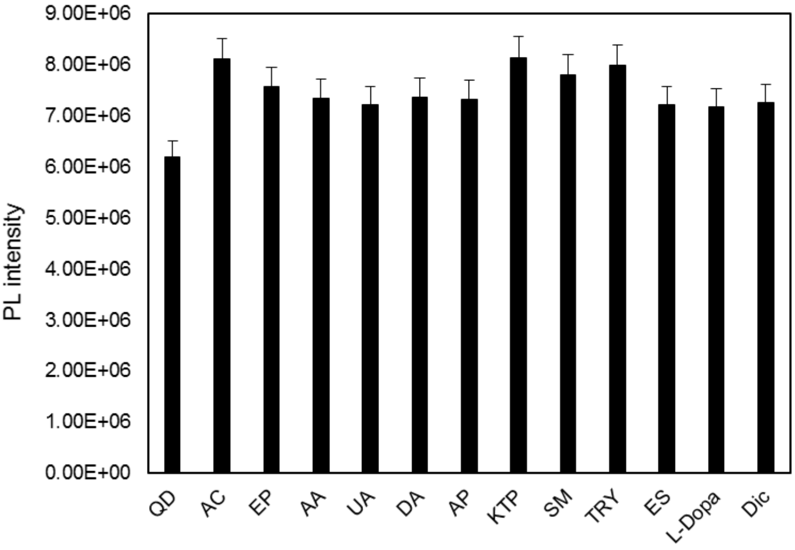


Figure 68: Effect of fixed concentration of 1.0×10^{-7} mol·L^{-1} of analytes on the fluorescence intensity of the CdSe/ZnS-L-cys QDs probe at 300 nm. Control= CdSe/ZnS-L-cys QD (1.0 mg/3.0 mL)

6.3.2 Triclosan sensing

6.3.2.1 Fluorescence properties of triclosan standard solution

The fluorescence behaviour of triclosan was recorded at different excitation wavelengths. The highest emission intensity was found at 407 nm (Figure 69a). Energy transfer is a possible mechanism for the fluorescence enhancement because there are overlapping bands between the absorption spectra of QDs and the emission spectra of triclosan in the range 350–500 nm (Figure 69b).

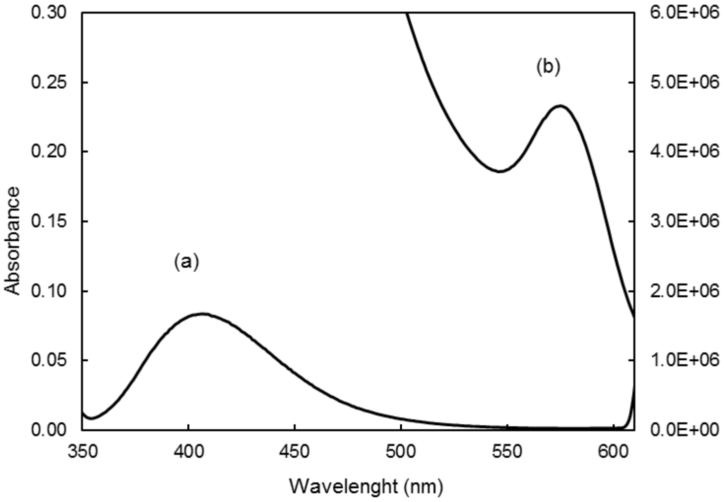


Figure 69: Florescence spectra of triclosan (1.4 × 10^{-5} mol·L^{-1}) at excitation wavelength 310 nm (a) and UV-Vis absorption spectra of CdSe/ZnS-GSH QDs (b)

6.3.2.2 Effect of aqueous CdSe/ZnS-GSH QDs concentration

The concentration of CdSe/ZnS-GSH QDs influences the fluorescence intensity and sensitivity of the system. Thus, different concentrations of CdSe/ZnS-GSH QDs (1.0 mg, 1.5 mg, 2.0 mg, 2.5 mg, 3.0 mg in 3.0 mL water) were prepared and 5.0 × 10^{-9} mol·L^{-1} of triclosan was added separately to each. F_0 was the fluorescence intensity of the QD in the presence of water. It was found that high concentrations of aqueous CdSe/ZnS-GSH QDs decreased the sensitivity of the system as self-quenching of QDs occurred (Figure 70) therefore the optimum concentration was 1.5 mg in 3.0 mL water.

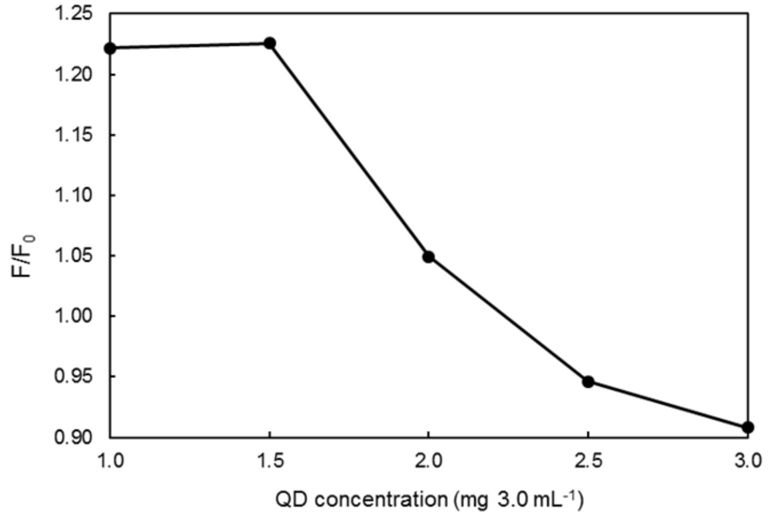


Figure 70: Effect of the concentration of aqueous CdSe/ZnS-GSH QDs in the presence of 5.0 × 10^{-9} mol·L^{-1} triclosan, fluorescence intensity without triclosan (F_0), fluorescence intensity with triclosan (F)

6.3.2.3 *Effect of incubation time*

The effect of reaction time on the fluorescence intensity of the GSH-capped CdSe/ZnS QDs-triclosan system was investigated at different time intervals in the presence of 7.0 × 10^{-8} mol·L^{-1} triclosan (Figure 71). The maximum was found after 5 min, which indicated that the reaction was completed within 5 min at room temperature. Further studies were thus carried out after 5 min.

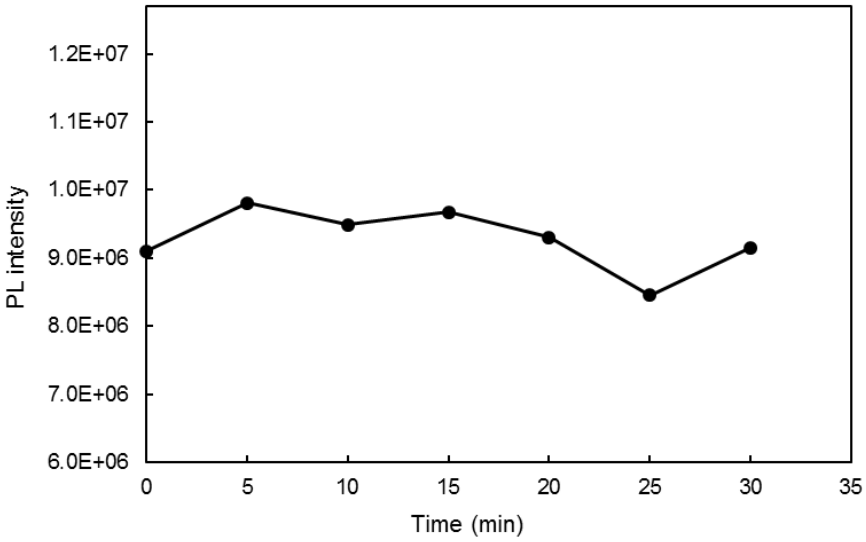


Figure 71: Effect of reaction time on the fluorescence intensity of GSH-capped CdSe/ZnS QD-triclosan system

6.3.2.4 *Fluorescence behaviour of GSH-capped CdSe/ZnS QDs in the presence of triclosan*

Under optimal condition, the fluorescence spectra of CdSe/ZnS-GSH QDs with different concentrations of triclosan were recorded. Within the concentration range of 1 × 10^{-8}–5 × 10^{-8} mol·L^{-1}, the intensity of aqueous CdSe/ZnS-GSH QDs enhanced with the introduction of triclosan (Figure 72).

6.3.2.5 *Calibration curve, LOD and LOQ*

Fluorescence intensity of GSH-capped CdSe/ZnS QDs increased linearly in the narrow concentration range of 1 × 10^{-8} mol·L^{-1} to 7 × 10^{-8} mol·L^{-1} of triclosan ($r^2 = 0.9885$) (Figure 72). In comparison, the worldwide concentration range of triclosan in water has been found to range from 1.4–40 000 ng·L^{-1} (5.0 × 10^{-12}–1.4 × 10^{-7} mol·L^{-1}) in surface waters, 20–86 161 ng·L^{-1} (7.0 × 10^{-11}–3.0 × 10^{-7} mol·L$^{-1)}$ in waste water influent, 23–5370 ng·L^{-1} (8.0 × 10^{-11}1.8 × 10^{-8} mol·L^{-1}) waste water effluent, <0.001–100 ng·L^{-1} (< 3.5 × 10^{-15}–3.5 × 10^{-10} mol·L^{-1}) in seawater (Dhillon et al., 2015).

The detection and quantification limits that were calculated using 3δ/m and 10δ/m, where δ was the standard deviation of the blank signal (n = 10) and m was the slope of the linear range, were found to be 3.0 × 10^{-9} (3.0 nM) and 10.0 × 10^{-9} mol·L^{-1} (10.0 nM) respectively. As mentioned in previous reports, the determination of triclosan has predominately been performed by chromatography-based methods and nanomaterial-based electrode systems using voltammetric methods. Although the best detection limit for triclosan was achieved by HPLC-UV detection (3.4 × 10^{-12} mol·L^{-1}) in tap water and river water (Kim et al., 2013a), this method has disadvantages in terms of analytical running costs, complexity, environmentally unfriendly solvent use, expensive instrumentation, long analysis time, as well as the need for highly skilled technicians and laborious sample pretreatment. It is therefore not ideal for routine screening analysis (Guo et al., 2009). Further work is planned to optimise the QD-based fluorescence sensor for triclosan.

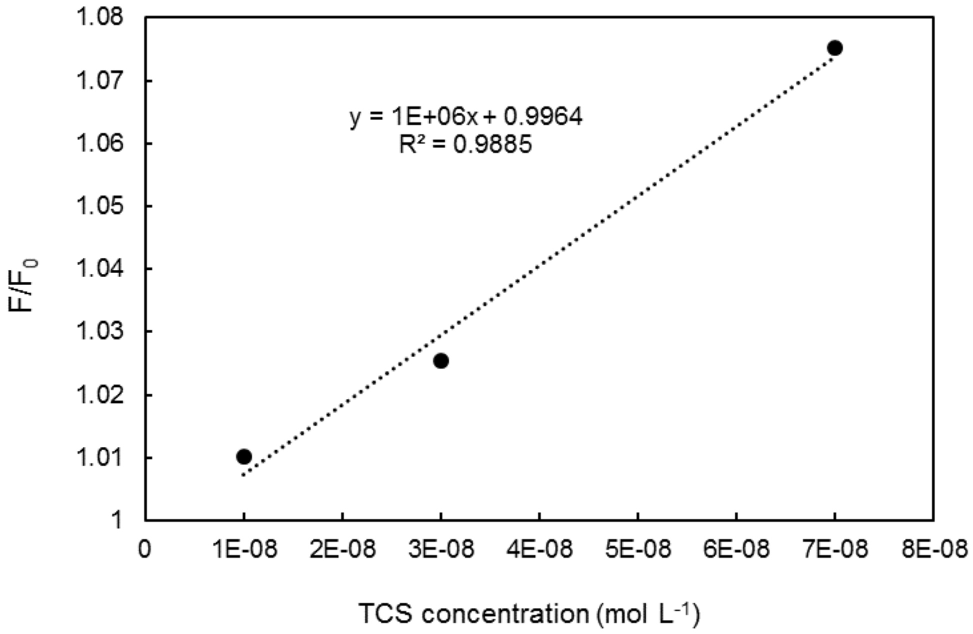


Figure 72: Linear graph of F/F$_0$ versus triclosan concentration based on detection using GSH-capped CdSe/ ZnS QDs

6.3.3 Pesticide sensing

The results in Figure 73, Figure 74 and Figure 75 show that for all the QD sensors tested, the fluorescence signal of the QD solutions (F$_0$) increased after adding the different concentrations of atrazine (F). This shows that atrazine was interacting with the QDs possibly through hydrogen bonding, which causes a "turn-on" PL effect. The hydrogen bonding allows for the atrazine molecules to be in close proximity with the QDs, allowing for energy transfer from the donor (atrazine) to the acceptor (QDs). This therefore leads to an increase in PL signal of the QDs. It was also observed that there was no significant shift in PL emission wavelengths for all the probe solutions which indicates their stability.

Figure 76, Figure 77, and Figure 78 show the relationship between F/F$_0$ ratios and atrazine concentration. It was observed that this ratio for the zinc-based probe (ZnSeS/ZnTe/ZnS QDs) did not follow a linear relationship with change in atrazine concentration (Figure 76), which indicated that it was not a suitable sensor for this application (possibly the energy transfer process was not efficient or there was surface activity on the QDs).

Promising results were obtained with the CdSe and CdTeSe/ZnSe/ZnS QD probes. CdSe QDs showed a linear increase of the F/F$_0$ ratio with increasing atrazine concentration from 1×10^{-7} mol·L^{-1} up to 4×10^{-7} mol·L^{-1} (Figure 77). The LOD was calculated from the regression curve and found to be 6.2×10^{-8} mol·L^{-1} (13.4 µg·L^{-1}). A similar observation was also observed with the CdTeSe/ZnSe/ZnS QD probe; however, the calibration point at 3×10^{-7} mol·L^{-1} appeared to be an outlier and was therefore excluded (Figure 6.17).

The LOD for this probe was calculated to be 1.7×10^{-7} mol·L^{-1} (36.4 µg·L^{-1}). These detection limits are higher than what may be required for real sample testing, given the low occurrence of atrazine in South African water systems. For example, monitoring studies between July 2012 and April 2013 in the Vals and Renoster catchments found atrazine to occur at much lower concentrations, from 0.06–0.35 µg·L^{-1}. (Dabrowski, 2015a). Therefore, further optimisation is required.

These promising initial results, however, provide a basis for sensor development with these QD systems. The CdTeSe/ZnSe/ZnS QD probe is, however, preferred over the CdSe QDs because the cadmium core is protected by the two zinc shells and it is therefore less toxic. Also, it exhibited excellent florescence properties with a more intense and narrower PL peak compared to CdSe QDs. Thus, the ongoing studies will focus on optimising the CdTeSe/ZnSe/ZnS QD probe for pesticide sensing, including the generation of replicate measurements to determine the precision of the method.

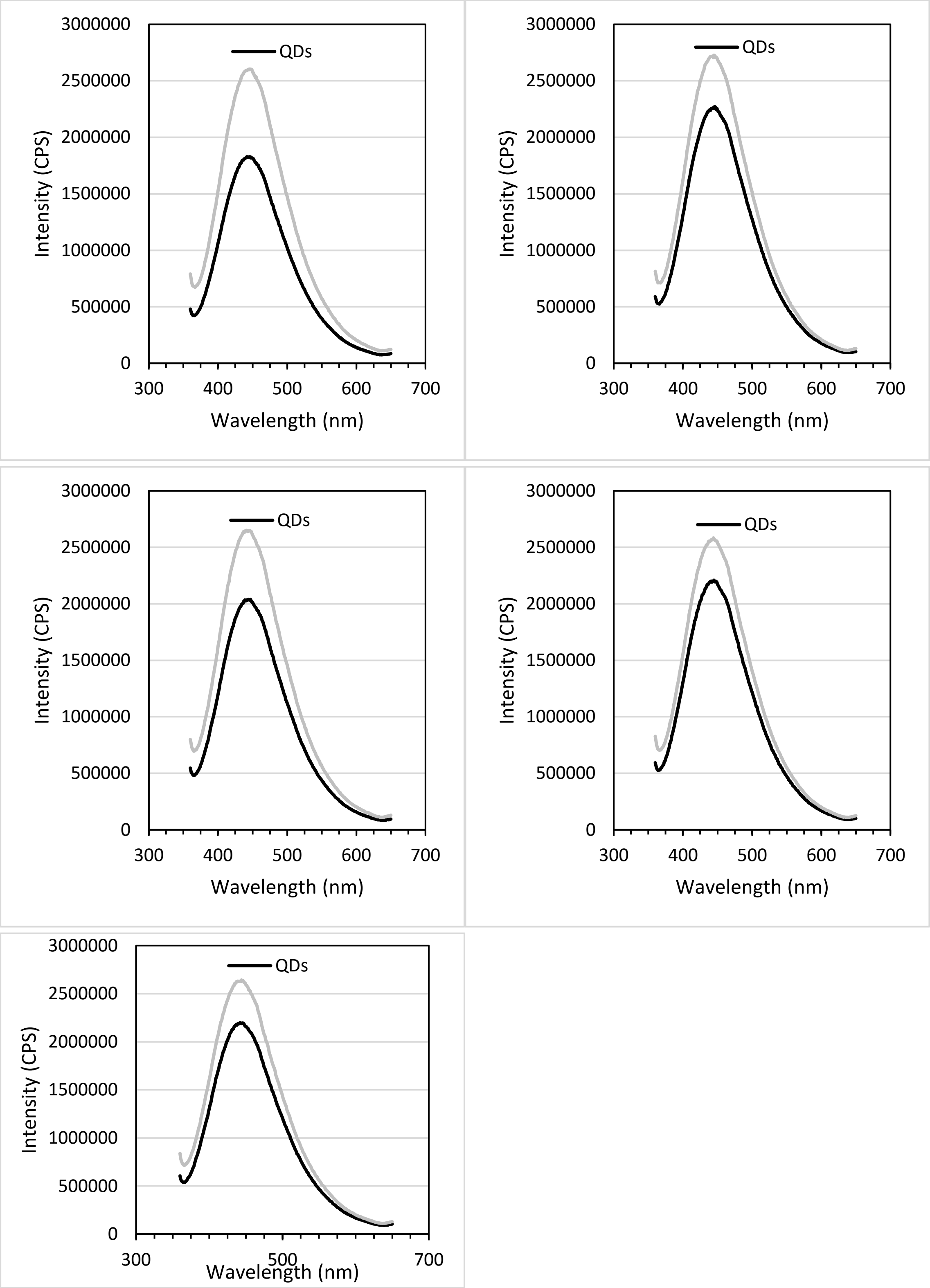


Figure 73: Fluorescence response of ZnSeS/ZnTe/ZnS QD sensor solution to difference concentrations of atrazine (0.1 µM–0.5 µM). In each case, 1000 µL of QD solution was spiked with 100 µL atrazine solution. A 5 min equilibration time was allowed after spiking. The excitation wavelength was 350 nm in all cases

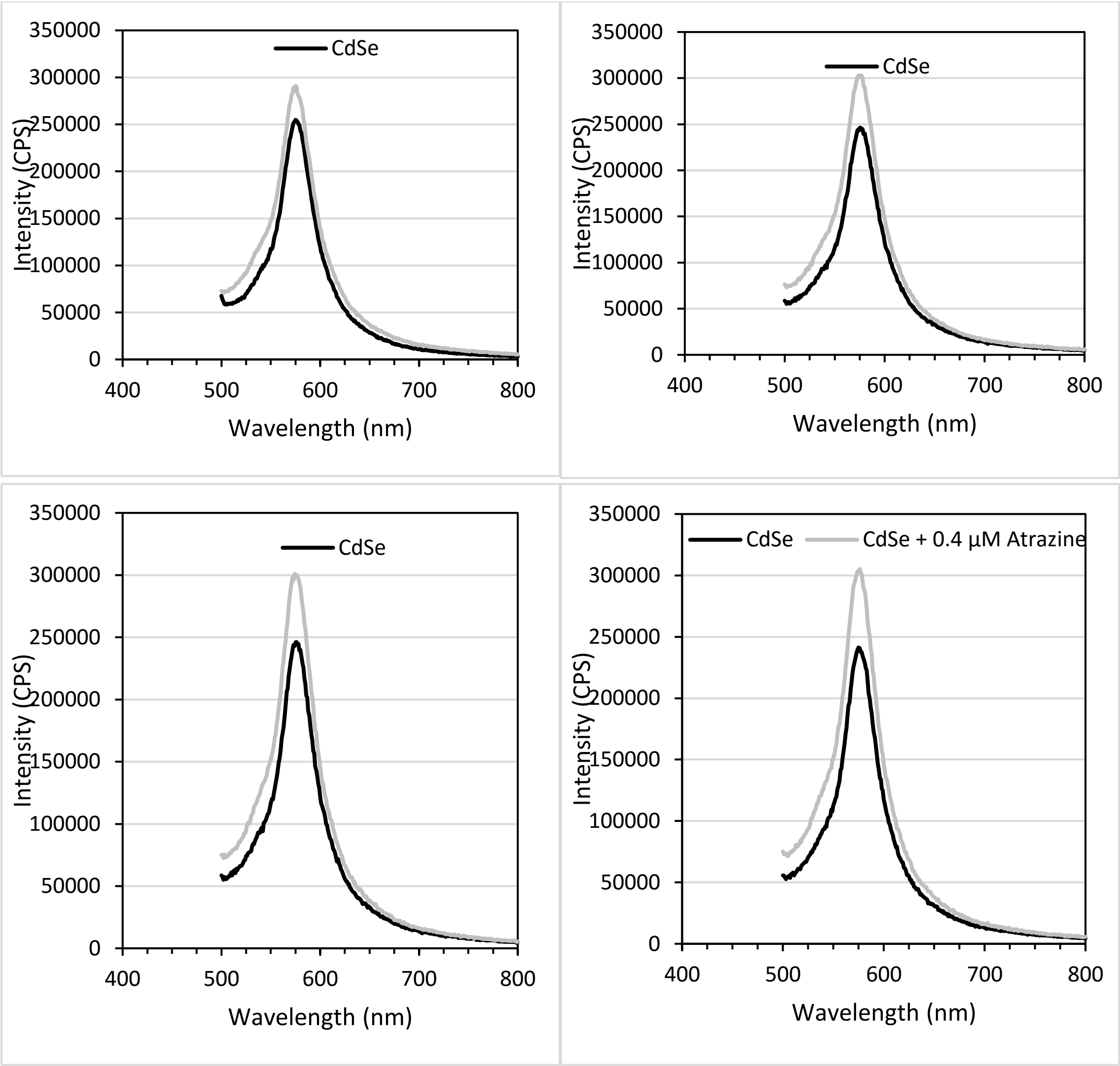


Figure 74: Fluorescence response of CdSe QD sensor solution to different concentrations of atrazine (0.1 µM–0.4 µM). In each case 1000 µL of QD solution was spiked with 100 µL atrazine solution. A 5 min equilibration time was allowed after spiking. The excitation wavelength was 490 nm in all cases

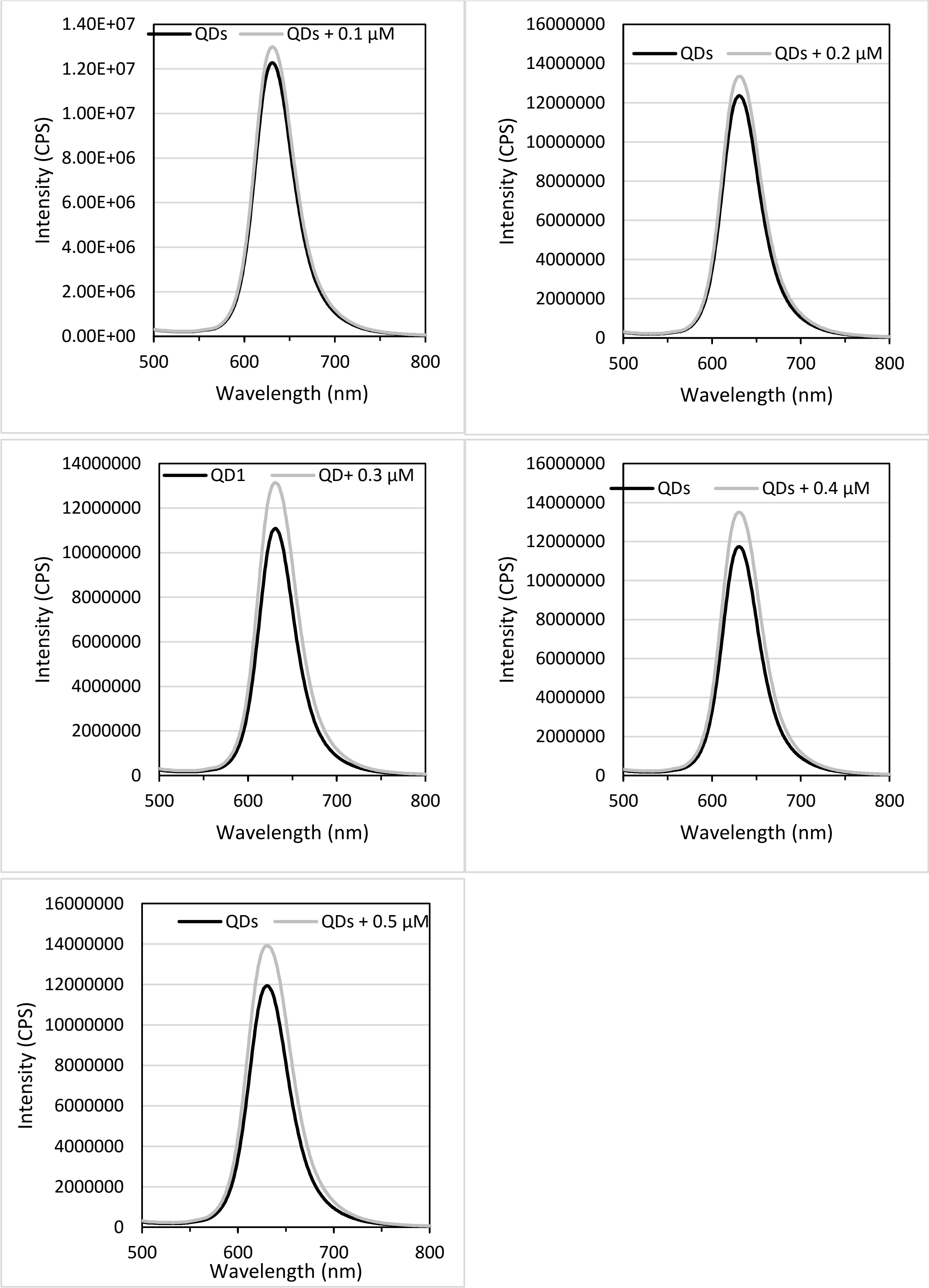


Figure 75: Fluorescence response of CdTeSe/ZnSe/ZnS QDs sensor solution to different concentrations of atrazine (0.1 µM–0.5 µM). In each case 1000 µL of QD solution was spiked with 100 µL atrazine solution. A 5 min equilibration time was allowed after spiking. The excitation wavelength was 470 nm in all cases

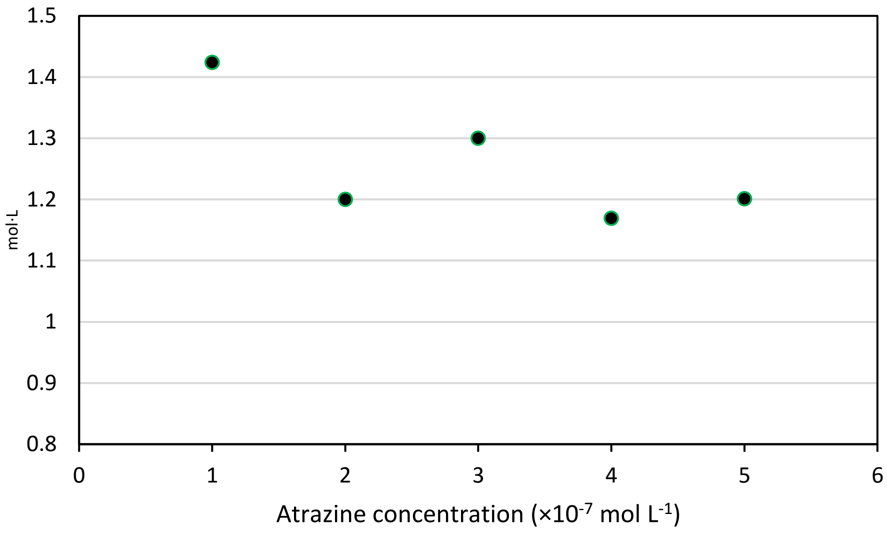


Figure 76: Calibration regression curve showing the non-linear response of ZnSeS/ZnTe/ZnS QDs in detecting atrazine in solution. F_0 is the fluorescence of the ZnSeS/ZnTe/ZnS QDs without the analyte and F is the fluorescence intensity after adding 100 µL of increasing concentrations of atrazine (0.1–0.5 µM) to the same QD solution

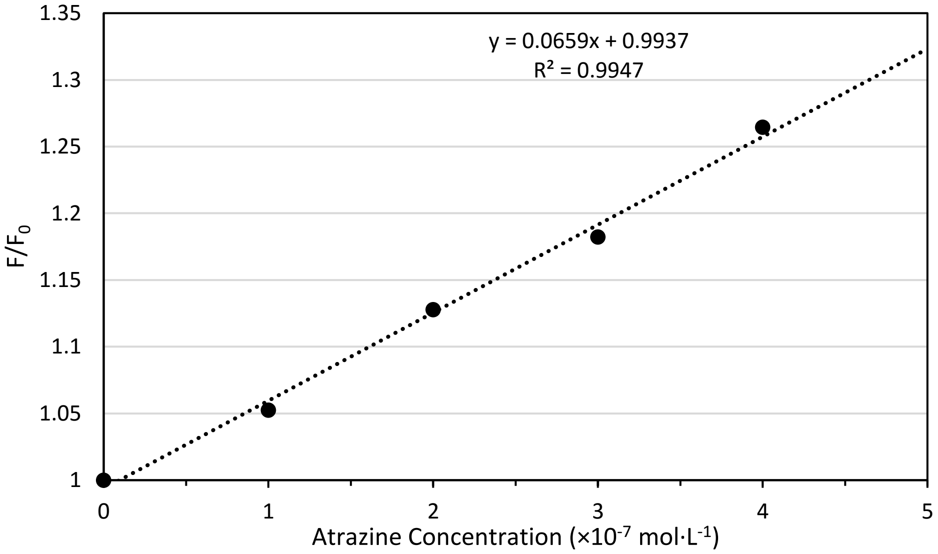


Figure 77: Calibration regression curve showing the linear response of CdSe QDs in the detection of atrazine in solution. F_0 is the fluorescence of the CdSe QDs without the analyte and F is the fluorescence intensity after adding 100 µL of increasing concentrations of atrazine (0.1–0.4 µM) to the same QD solution

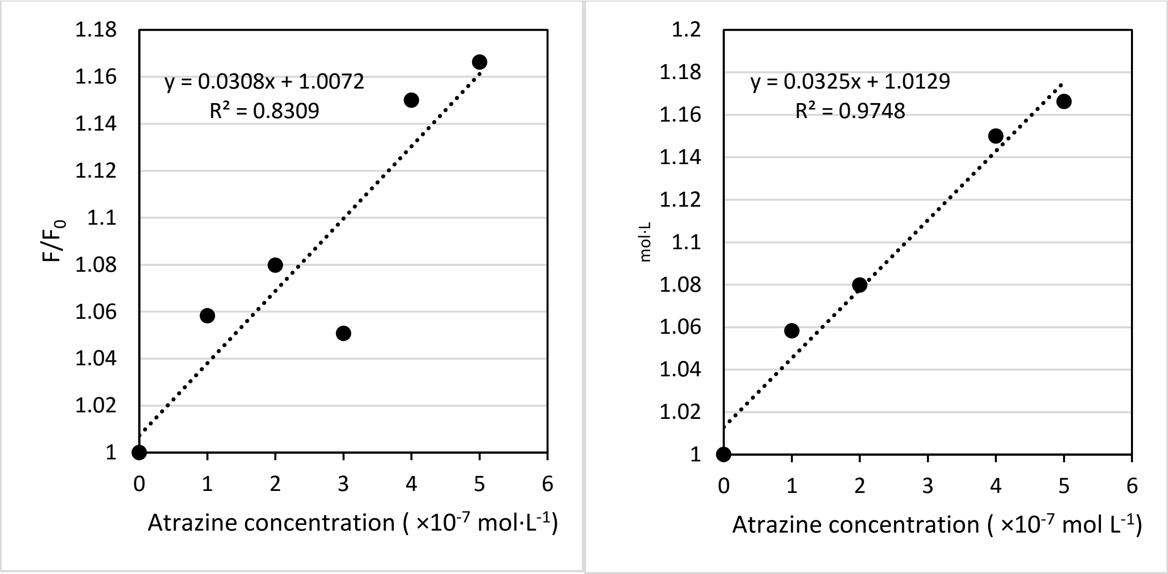


Figure 78: Calibration regression curve showing the linear response of CdTeSe/ZnSe/ZnS QDs in the detection of atrazine in solution. F_0 is the fluorescence of the CdTeSe-ZnSe-ZnS QD solution without the analyte and F is the fluorescence intensity after adding 100 µL of increasing concentrations of atrazine (0.1–0.5 µM) to the same QD solution

6.3.4 PAHs sensing

Figure 79 shows the effect of different PAHs at a fixed concentration (1×10^{-7} mol·L^{-1}) on the fluorescence intensity of the GQDs probe. Phenanthrene had the lowest quenching effect on the probe compared to the other PAHs that were investigated. It was selected for further sensing studies as a worst-case scenario of the PAHs considered, as results are expected to be enhanced (lower LODs) for the other PAHs, which will be tested further in due course.

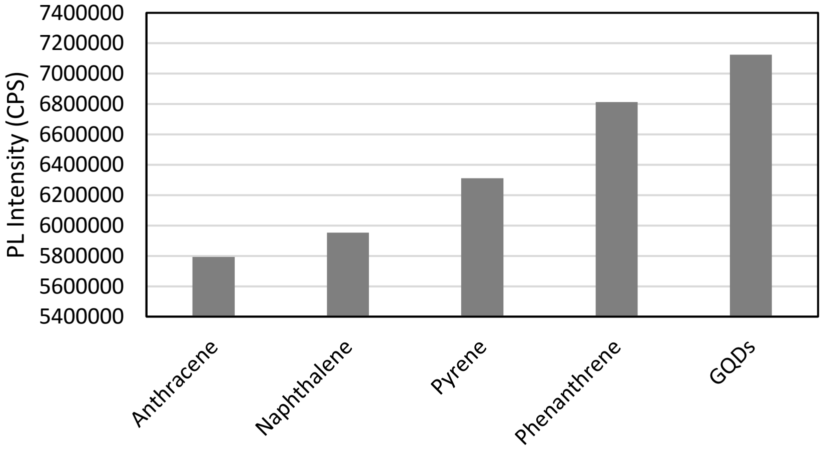


Figure 79: Effect of fixed concentration (1×10^{-7} mol·L^{-1}) of PAHs on the fluorescence intensity of GQDs (λex = 340 nm)

Figure 80 shows the fluorescence signal response of the GQDs as the phenanthrene concentration was changed from 1–5 × 10^{-7} mol·L^{-1}. Signal quenching of GQDs upon interaction with aromatic compounds could be due to FRET from the GQDs to the PAH molecules or charge transfer. A study by Fan et al. (2012) with TNT (2,4,6-trinitrotoluene) showed that FRET was the more likely mechanism for this analyte with GQDs. Fluorescence lifetime measurements have to be carried out in order to propose a plausible mechanism in the case of the PAHs. Figure 80 shows that the signal decreased linearly with increasing phenanthrene concentration ($R^2 = 0.9572$). Thus, the GQDs probe can potentially be used as a sensor to detect phenanthrene and possibly other PAHs in water.

The LOD was calculated using the equation 3δ/K, where K is the slope of the calibration graph and δ is the standard deviation of blank measurements (n = 9). It was found to be 2.5 × 10^{-8} mol·L^{-1} (4.5 µg·L^{-1}) for phenanthrene. This shows great potential for the sensor for detecting the low concentrations of PAHs reported occur in South African water systems. Chimuka et al. (2015) reported that phenanthrene occurs in local river water between 0.119–0.197 µg·L^{-1}, suggesting that improvements have to be made to better the sensitivity of our sensor or that pre-concentration of the sample would be required (as is done for standard GC–MS methods).

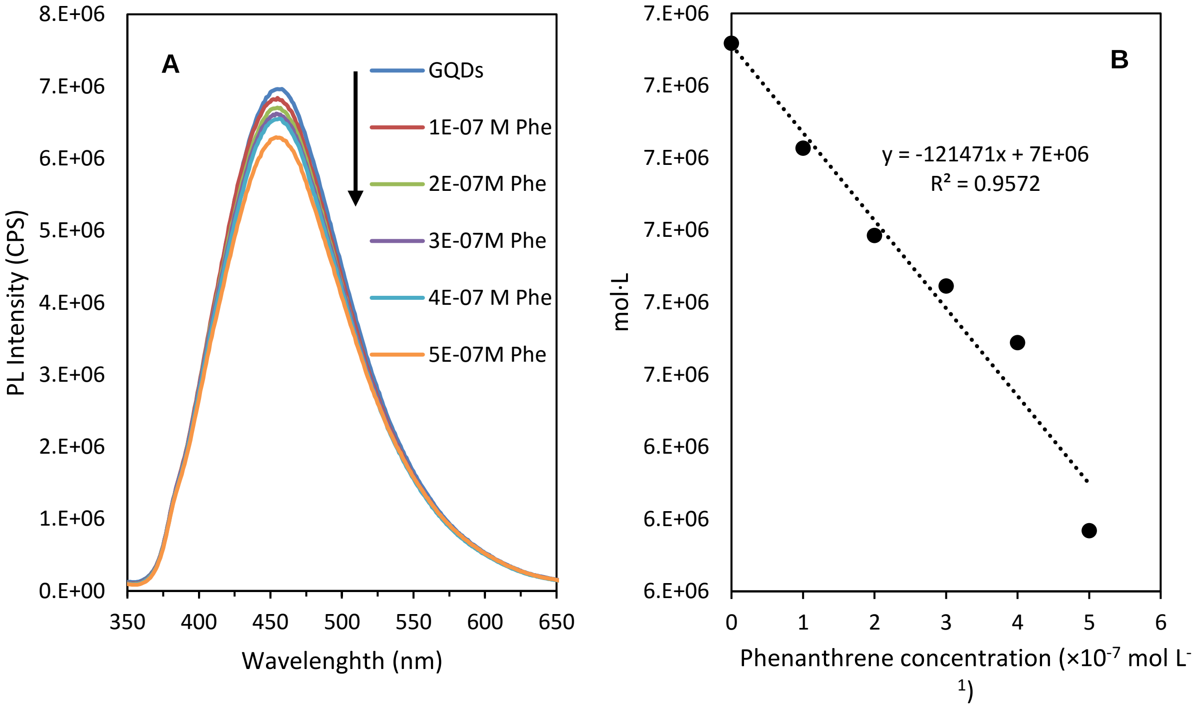


Figure 80: (a) Fluorescence detection of phenanthrene at increasing concentrations corresponding to a decrease in PL signal of the GQDs. Phenanthrene concentrations were 1–5 × 10^{-7} mol·L^{-1}. Excitation was at 340 nm for all measurements. (b) Calibration regression curve showing a linear response of the GQDs upon interaction with phenanthrene

Application of GQDs as a sensor for the other PAH compounds (pyrene, anthracene, and naphthalene) did not give satisfactory results as shown in Figure 81, Figure 82 and Figure 83. In these cases, there was no appreciable change in PL intensity of the GQDs probe with increase in PAH concentration. Thus, a linear relationship between PL intensity and concentration was not established for these PAHs. The ongoing work in this regard is investigating possible causes and testing different concentration ranges.

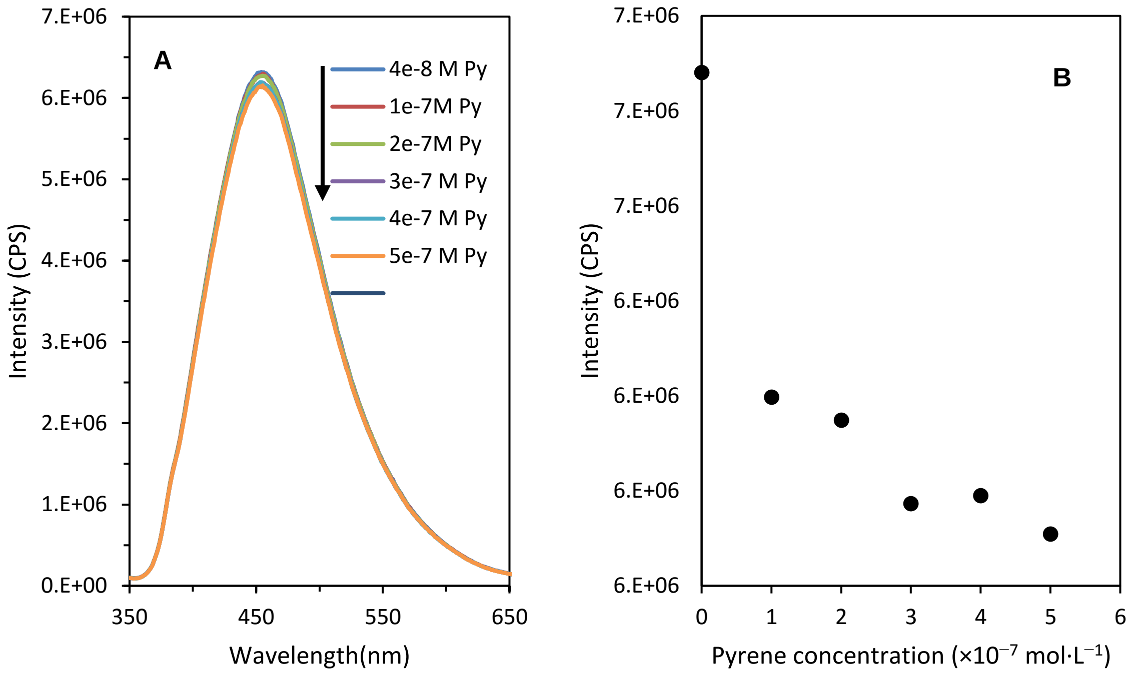


Figure 81: (a) Fluorescence detection of pyrene at increasing concentrations corresponding to a decrease in PL signal of the GQDs. Pyrene concentrations were 1–5 × 10^{-7} mol·L^{-1}. (b) Regression curve showing response of the GQDs upon interaction with pyrene

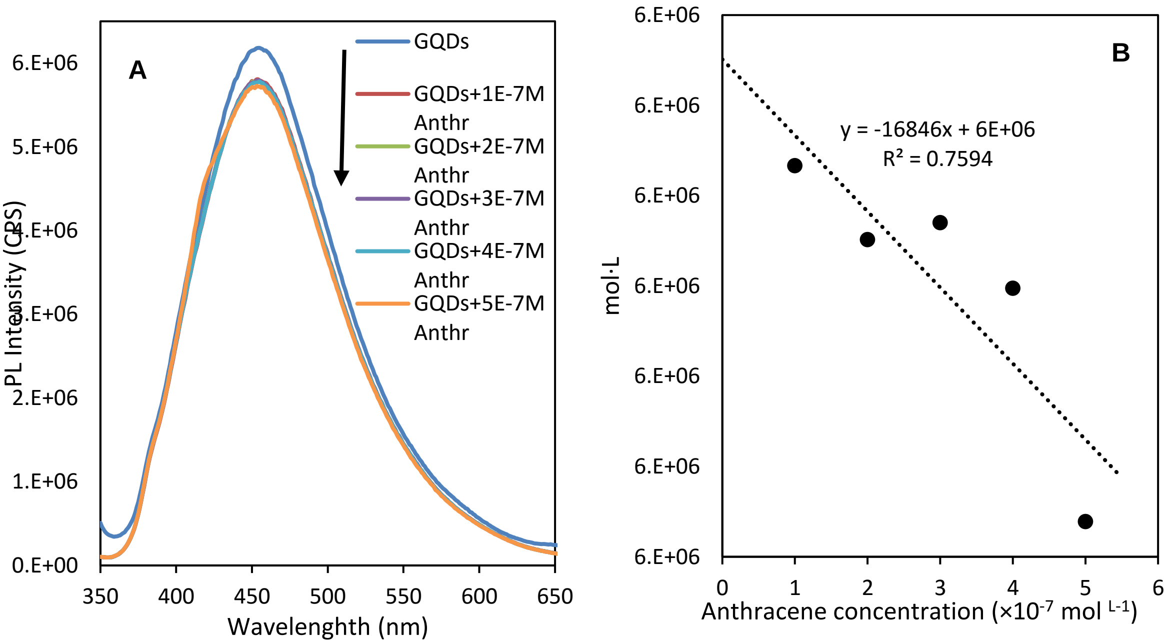


Figure 82: (a) Fluorescence detection of anthracene at increasing concentrations corresponding to a decrease in PL signal of the GQDs. Anthracene concentrations were 1–5 × 10^{-7} mol·L^{-1}. (b) Regression curve showing response of the GQDs upon interaction with anthracene

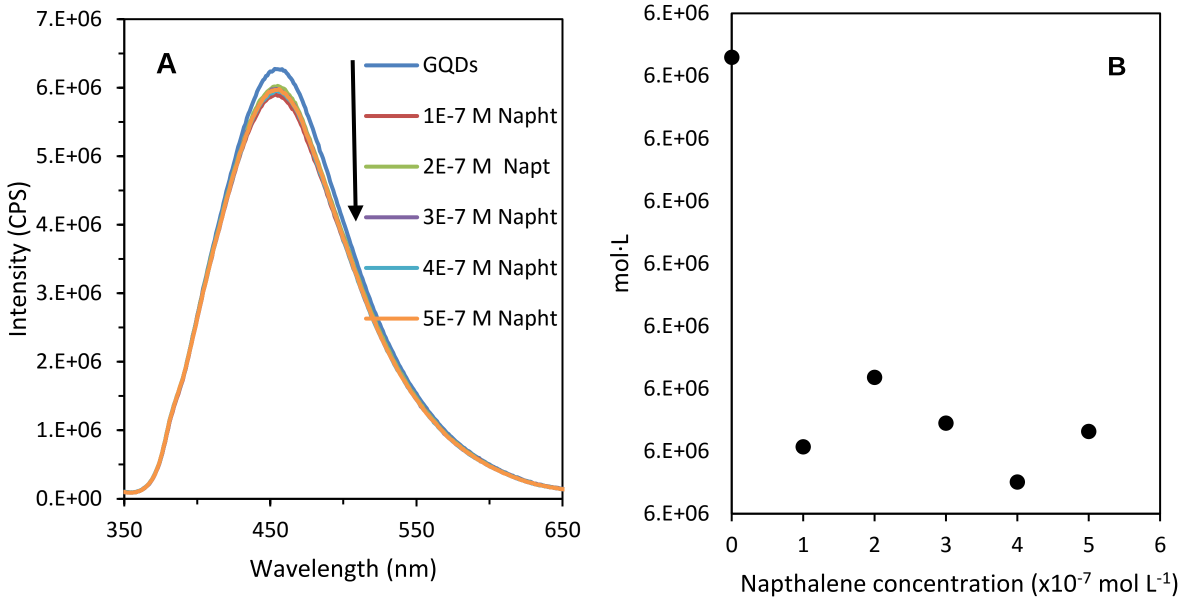
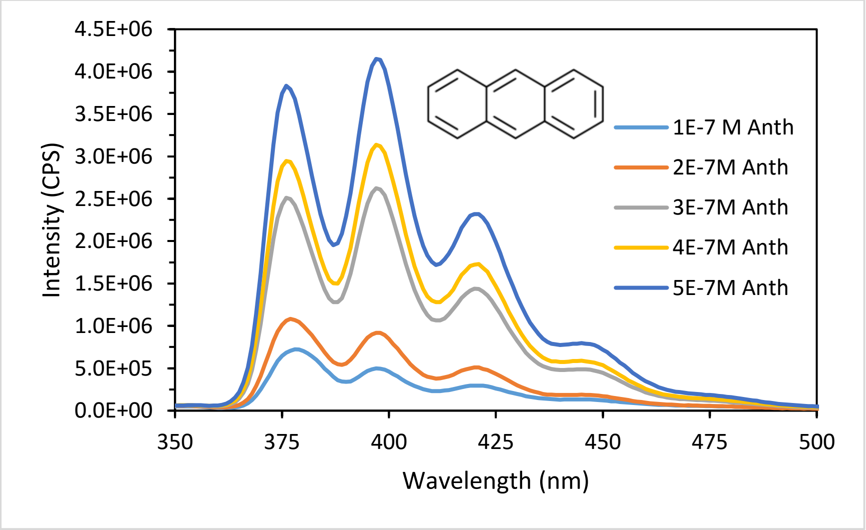


Figure 83: (a) Fluorescence detection of naphthalene at increasing concentrations corresponding to a decrease in PL signal of the GQDs. Naphthalene concentrations were 1–5 × 10^{-7} mol·L^{-1}. (b) Regression curve showing response of the GQDs upon interaction with naphthalene

6.3.4.1 Fluorescence properties of PAH standard solutions

The fluorescence properties of the different PAHs alone were studied to try to understand their possible effects on the PL spectra of the GQDs during sensing. Characteristic spectra for the different PAHs were observed and are shown in Figure 84, and these are dependent on the excitation wavelength that was used. For phenanthrene, the emission wavelength is around 335–400 nm, and this could possibly explain the small shoulder on the emission peak.

For each PAH compound, the maximum emission peak was also plotted against concentration to study the effect of concentration on the fluorescence intensities (Figure 85). Pyrene and anthracene showed a linear response while phenanthrene and naphthalene showed non-linear patterns. Phenanthrene shows possible self-quenching at higher concentration while the pattern observed for naphthalene is possibly due to volatilization losses.



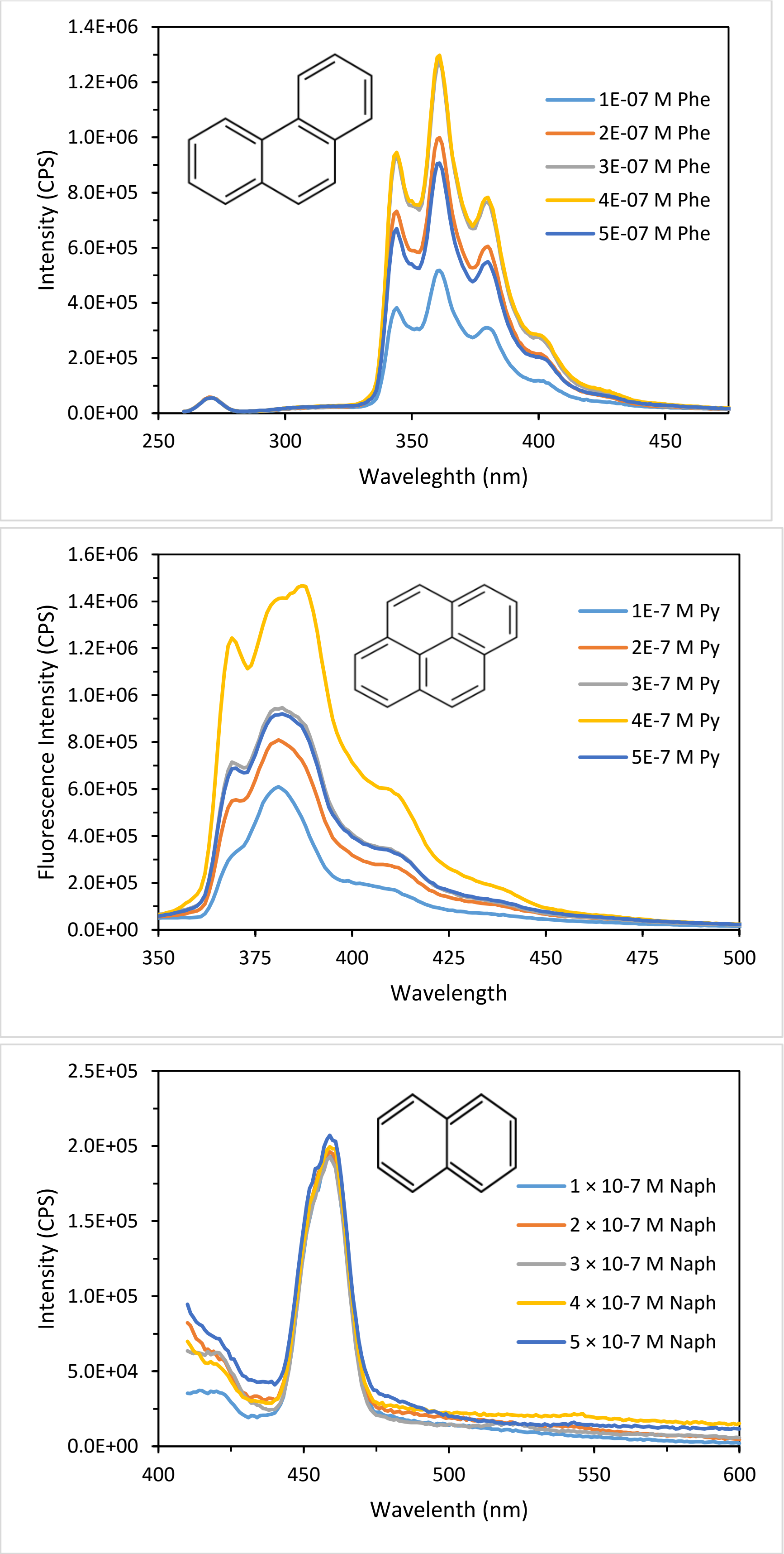
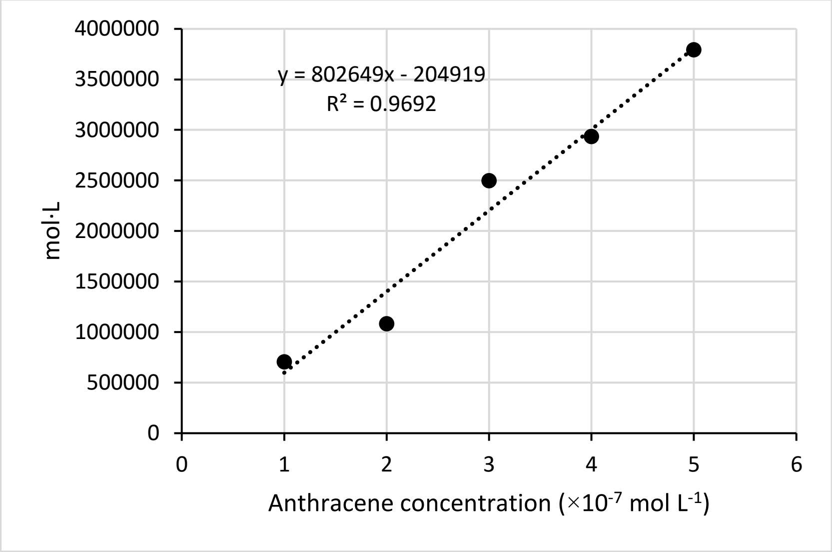
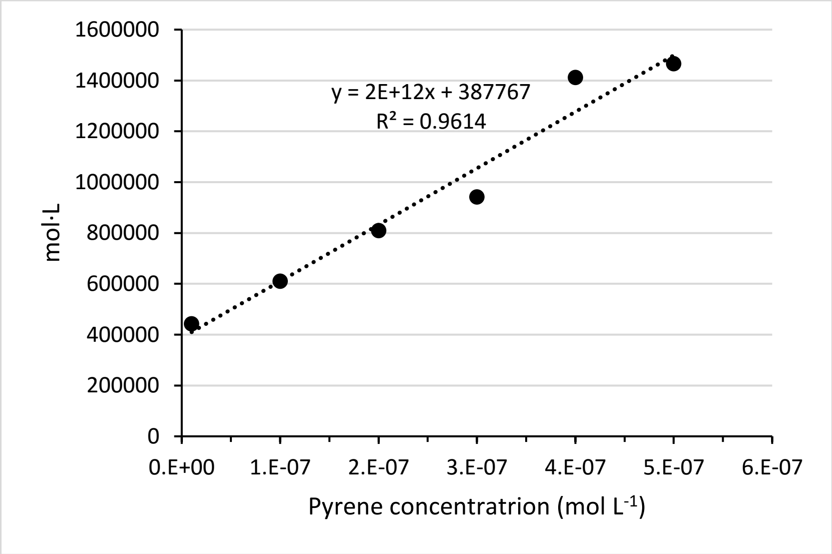
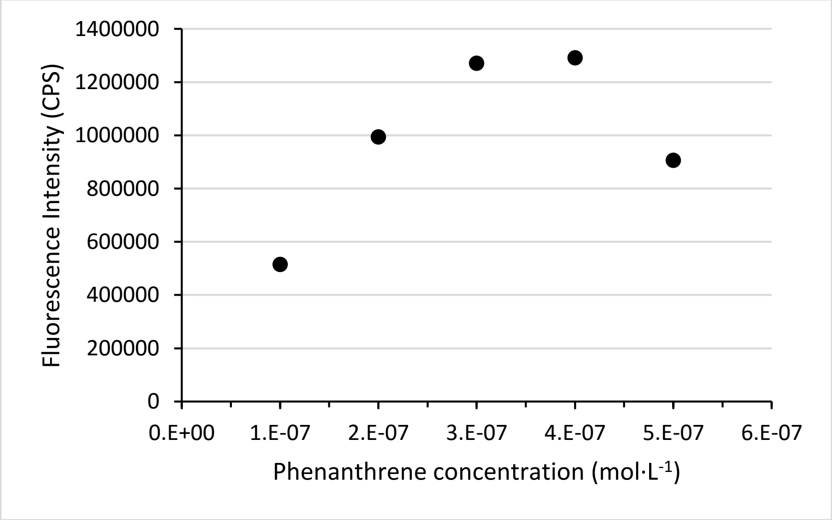


Figure 84: Fluorescence intensity spectra of the different PAHs at different concentrations. The excitation wavelength for anthracene and pyrene was 340 nm, 400 nm for naphthalene, and for phenanthrene it was 250 nm


4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
0
1
2
3
4
5
6
mol·L
y = 802649x - 204919
R² = 0.9692
Anthracene concentration (×10-7 mol L-1)


1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
0.E+00
1.E-07
2.E-07
3.E-07
4.E-07
5.E-07
6.E-07
mol·L
y = 2E+12x + 387767
R² = 0.9614
Pyrene concentratrion (mol L-1)


1400000
1200000
1000000
800000
600000
400000
200000
0
0.E+00
1.E-07
2.E-07
3.E-07
4.E-07
5.E-07
6.E-07
Fluorescence Intensity (CPS)
Phenanthrene concentration (mol·L-1)

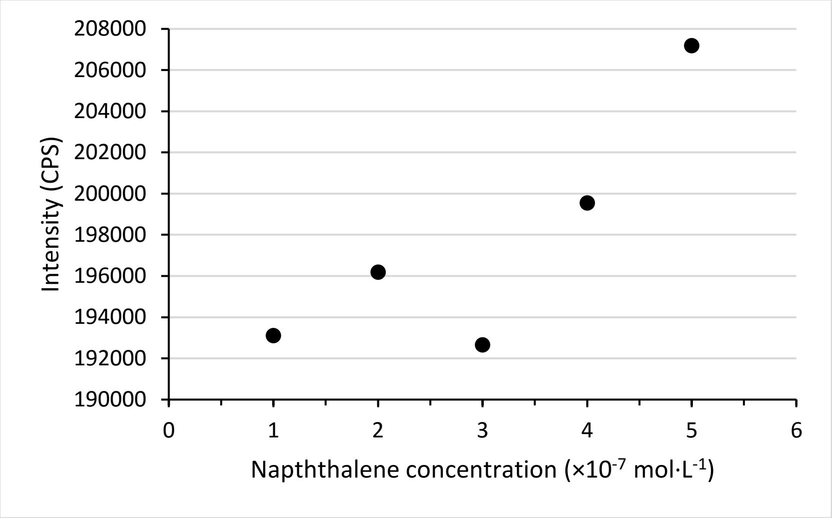


Figure 85: Direct fluorescence measurements of the as-prepared PAH standards in the 1–5 ×10^{-7} mol·L^{-1} concentration range

6.3.4.2 GQDs sensor optimisation

Sensor optimisation is essential to improve and validate the performance of the sensor. Firstly, the effect of interaction time (5–45 min) between the GQDs sensor and phenanthrene solution before taking PL measurements was investigated (Figure 86). Longer interaction times were observed to have a quenching effect on the PL signal. To investigate this further, the stability of the GQDs within the same time range was investigated without any PAH analyte. The GQDs solution was found to be also unstable and exhibiting self-quenching, but there was an additional quenching effect in the presence of phenanthrene up to 30 min, which indicates that the GQDs still hold potential as sensors in this regard. Doping the GQDs with nitrogen atoms could be one way of improving their photostability.

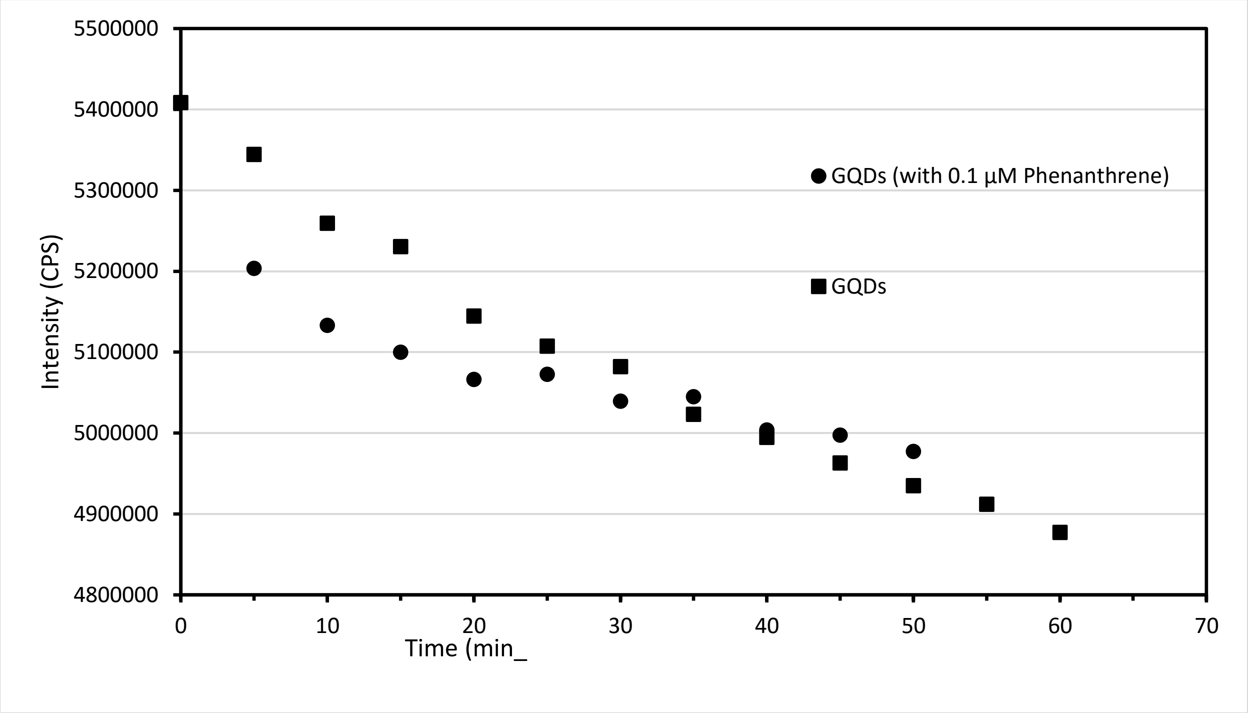


Figure 86: Effect of equilibration time on the quenching of GQDs signal by 1×10^{-7} M phenanthrene (blue). PL stability of GQDs in the absence of PAH analyte (red) showing possible self-quenching and photo-instability

6.4 Quantum dot immobilisation

Figure 87 shows the PDMS-QD films that were produced by spin-coating on microscope glass slides. Films with QDs had a slight brownish tint compared to the control, which was pure PDMS. This brownish tint comes from the colour of the QDs. Fluorescence analysis (Figure 88) showed that the control had no fluorescence emission, as expected and that only the PDMS-QDs films had fluorescent properties.

The PL peaks were, however, very broad and had greatly reduced intensities compared to the pure QDs in solution. This could be due to poor dispersion (Eita et al., 2012) of the QDs in the polymer matrix or the concentration of the QDs on the matrix was simply too low. This was noted from the fact that the thickest film, which was not spin-coated, had the most intense PL signal (Figure 88). Thus, optimisation of these parameters needs to be carried out in order to improve the PL properties of the PDMS-QD films before they can be applied for target analyte sensing. Also, full characterisation of the materials is necessary in order to understand how the QDs have interacted with the PDMS, and means to better remove small bubbles in the films needs to be investigated.

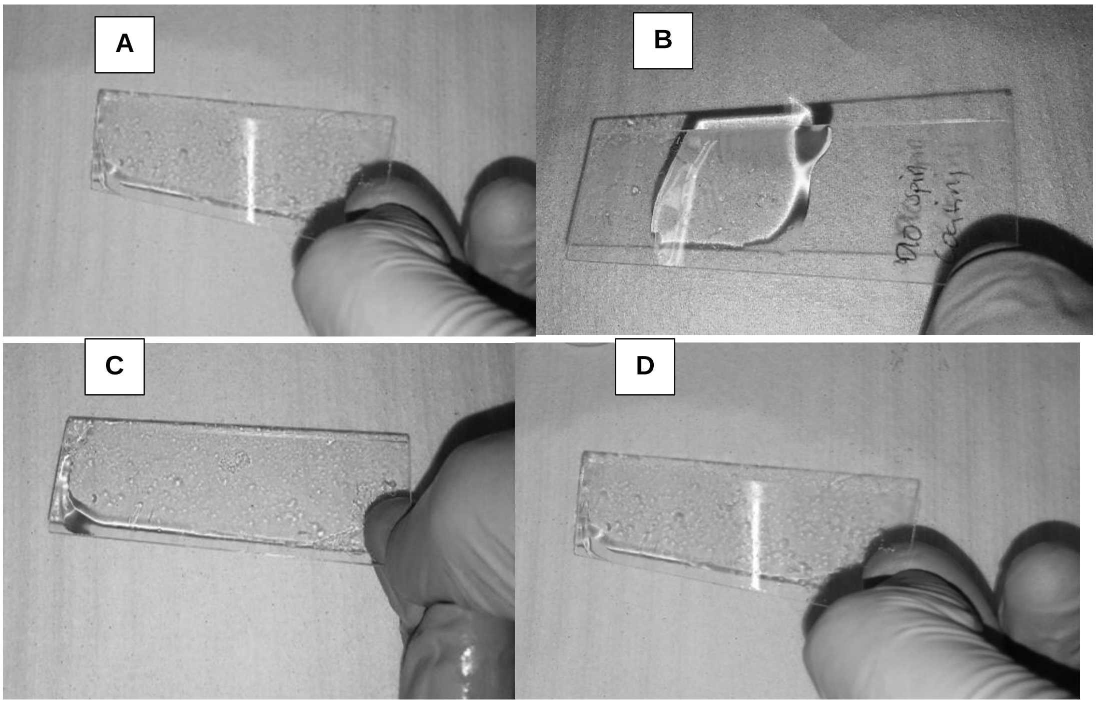


Figure 87: Glass slides with films of PDMS with immobilised CdSeTeS QDs. A: pure PDMS film (control), B: QD polymer matrix without spin-coating, C: single layer film, D: double-layer film

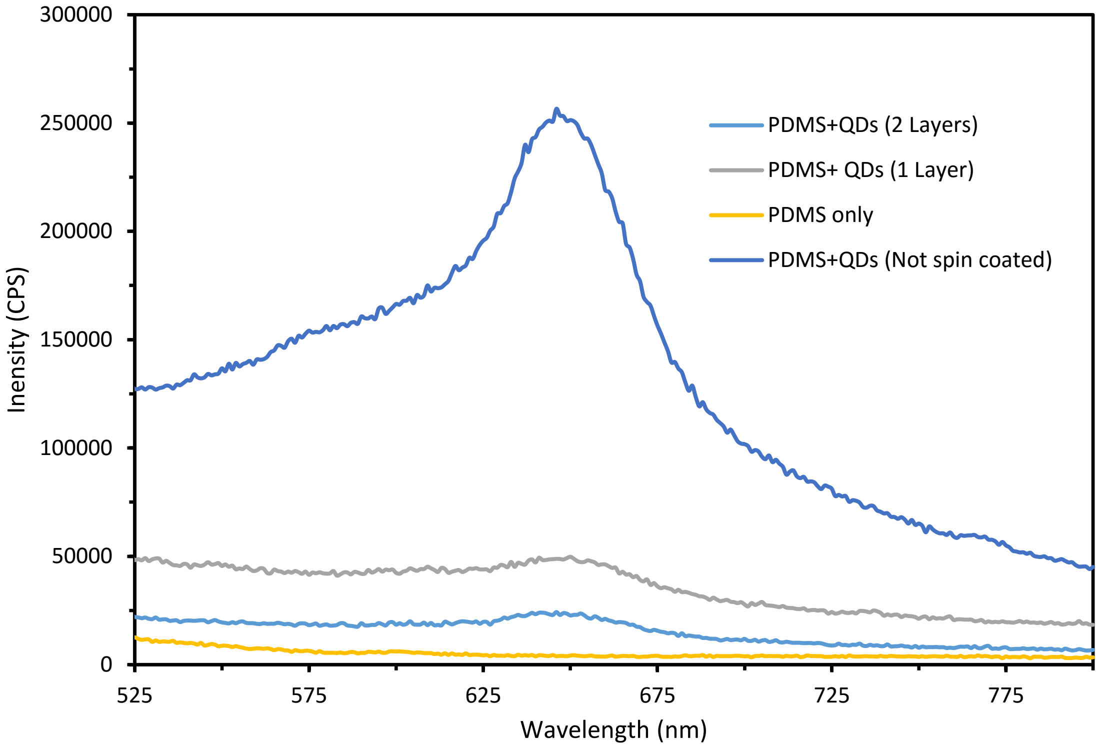


Figure 88: Overlaid fluorescence spectra of PDMS and PDMS-QD films on glass slides showing the QD PL emission peak at 648 nm. The control showed no fluorescence peak at this wavelength

6.5 Cost Benefit Analysis for Quantum Dot Fluorescence Sensors

QD-based fluorescence sensors have emerged as an alternative to conventional methods. Affordable analytical screening methods are desirable for routine monitoring of ECPs in water systems, especially for a developing country like South Africa where resources are scarce. Some of the benefits of moving towards using QD sensors for detection of pollutants including ECPs have been discussed in detail in previous chapters of this report and are briefly:

- **Sensitivity:** QD sensors have been shown in various studies to offer ultra-low detection limits of various pollutants compared to other analytical methods.
- **Versatility:** QDs are tunable materials that can be functionalised using different approaches to design selective probes for specific target analytes. This therefore allows them to be tailored for a wide range of ECP compounds.
- **Stability:** QDs have excellent stability and can survive several months under room temperature storage without significant change in their fluorescence properties. This long shelf life allows for their application in studies where, for instance, seasonal monitoring is being undertaken.
- **Less labour-intensive:** Using QD probes requires less sample preparation than chromatography-based methods.
- **Cost-effective:** Finally, the costing analysis that is presented in this report shows that the cost of producing and using QD probes is far less than that of analysis of water samples by conventional methods. Also, use of the probes does not require as expensive equipment or consumables.
- **Reusability:** By immobilizing the QDs in a suitable solid matrix, it is possible to reuse the fluorescence sensor after removal of the analyte(s) by solvent rinsing.

Table 23 details the costs involved in producing a single batch of two different types of QDs used in this study for comparison purposes. The cost per batch was calculated from the supplier's price and the amount used during synthesis in each case. Since QDs fluoresce even at very low concentrations, a single batch can be used to analyse numerous samples over different days and months – because of their stability – making them economically favourable over conventional methods. For example, producing about 2 g of L-cys-CdSeTe/ZnSe/ZnS QD-GO nanocomposite costs about R1689 (Table 23), and only 0.7 mg is required for each sensing analysis. This means that numerous samples can be analysed with this batch, making it cheaper than, for example, GC–MS, which costs around R2000 per sample (for an untargeted analysis where a range of ECPs is determined).

It should be noted that a number of items have been excluded from this first estimate costing exercise, namely, those associated with immobilisation (reagents and hardware); instrumentation (heating mantle and fluorescence spectrophotometer) and analyte standards used for calibration.

Table 23: Cost analysis for the production of two QDs sensors: L-cys-CdSeTe/ZnSe/ZnS QD-GO and GSH-CdSe QDs. Values in red are estimated quantities, while values in black are actual quantities

Category	Description	Cost per batch (R)	Cost per batch (R)
		L-cyst-CdSeTe/ ZnSe/ZnS QDs-GO	CdSe-GSH QDs
Solvents	Acetone	6	6
	Methanol	2	2
	Chloroform	7	8
	Ethanol	148	30
	Hydrochloric acid (HCl) 32%	3	0
	Sulphuric acid (H_2SO_4) 99%	7	2
	Hydrogen peroxide (H_2O_2) 30%	5	1
	Oleic acid (OA)	560	44
	Octadec-1-ene (ODE)	86	83
Powders	Cadmium oxide (CdO)	10	10
	Selenium powder (Se)	3	3
	Zinc oxide (ZnO)	2	2
	Sulphur (S)	0	0
	Tellurium powder (Te)	38	0
	Zinc diethyldithiocarbamate (ZDC)	7	0
	Zinc chloride	0	0
	TOPO	102	102
	Sodium nitrate ($NaNO_3$)	19	0
	Potassium hydroxide (KOH)	17	34
	N-Hydroxysuccinimide (NHS)	3	0
	N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride	41	0
Surface ligands and conjugation	N-acetyl-L-cysteine	0	32
	L-cysteine	19	39
	Glutathione	0	355

Category	Description	Cost per batch (R)	Cost per batch (R)
		L-cyst-CdSeTe/ ZnSe/ZnS QDs-GO	CdSe-GSH QDs
Graphene conjugation	Graphite powder	1	–
	Permanganate ($KMnO_4$)	11	–
	N,N-Dimethylformamide (DMF)	0	–
Other laboratory consumables	Centrifuge tubes	24	–
	Posture pipets	18	–
	Gloves, Pipette tips, vials, etc.	500	500
	Argon gas	50	50
		1689	1301

Table 24 compares a number of different analytical methods, including QD probes, for analysis of a range of organic compounds (including SVOCs and VOCs) and lists the limitations that are associated with each method.

Table 24: Comparison of QD fluorescence sensor method with other conventional methods. Data for other methods is adapted from (US EPA, 1997), which focused on VOCs in air

Technique	Analytes	Data Quality Level #	Analysis Time	Approximate Cost per Sample	Skill Level	Limitations
Detector tubes	> 100 specific compounds	1A/1B	5 to 15 min	R120 to R405 *	Low	High degree of cross-reactivity
Fibre optic chemical sensors	VOCs and SVOCs ≥ C_6	1A/1B	3 to 5 min	< R15 to R150 *	Low	Does not measure specific constituents
Colorimetric test kits	Aromatic hydrocarbons	1A/1B	10 to 20 min	R255 to R630 *	Low–Medium	Colours may be difficult to distinguish
TOV methods with FID/PID	Total VOCs	1A/1B	1 to 30 min	< R15 to R150 *	Low–Medium	Does not measure specific constituents
Immunoassay test kits	TEX/PAHs/TPH	1B	30 to 45 min	R300 to R900 *	Low–Medium	Cross-reactivity may affect interpretation
Field GC	Specific VOCs and SVOCs	2	10 to 60 min	R200 to R1050 *	Medium–High	Requires a skilled technician
GC–MS	> 100 compounds	2		R2000 to R3000	High	High running costs, extensive sample preparation
QD fluorescence sensors	> 100 compounds (e.g. ECPs: PAHs, pesticides, pharmaceuticals)	1B/2	5 to 10 min	R20 to R30	Low–Medium	Potential toxicity, possible cross-reactivity if not suitably functionalised

= 1A: Qualitative screening, 1B: Semi-quantitative screening, 2: Quantitative analysis

* Values from 1997, converted to Rand using ($1 = R15)

Abbreviations: **VOCs**: volatile organic compounds; **SVOCs**: semi-volatile organic compounds, **TPH:** total petroleum hydrocarbons; **TOV**: total organic vapour, **FID**: flame ionization detector; **PID**: photo-ionization detector; **GC–MS**: gas chromatography – mass spectrometry.

6.6 Conclusions and Future Outlook

Water-soluble L-cys and GSH functionalised CdSe/ZnS QDs were used as fluorescence sensors for the determination of acetaminophen and triclosan, respectively. The operating mechanism of the designed probes was based on the fluorescence "turn-on" mode. Under optimum conditions, with the addition of acetaminophen and/or triclosan to QDs, the original PL intensity of QD enhanced most likely due to fluorescence resonance energy transfer from analytes to QDs. The calibration plots were linear for both analytes and the methods could be applied to detect acetaminophen and triclosan at nanomolar levels. Other advantages of this method include simplicity, as well as stability and high resistivity of QDs against photobleaching (Dubertret et al., 2002).

Atrazine sensing experiments were conducted using different QDs as probes. Results show that interacting the QDs with atrazine leads to an enhancement of fluorescence signal, suggesting that a "turn-on" probe can be developed. CdTeSe/ZnSe/ZnS QDs were chosen as the most suitable probe for further optimisation because of their intense fluorescence signal and reduced toxicity. The LOD for this probe was 1.7×10^{-7} mol·L^{-1} (36.5 µg·L^{-1} range) with a 1–5 × 10^{-7} mol·L^{-1} linear range.

Sensing studies for selected PAHs were carried out using GQDs as sensor material. Interacting GQDs with PAHs resulted in quenching of the fluorescence signal as the PAH concentration increased. Good results were obtained with phenanthrene, with the probe showing a detection limit of 2.5×10^{-8} mol·L^{-1} with a 1–5 × 10^{-7} mol·L^{-1} range. A non-linear response was observed with the other PAHs tested (anthracene, pyrene and naphthalene), which are currently being investigated. In addition, a synthetic route to couple L-cys-CdSeTe/ZnSe/ZnS QDs to graphene to form a stable novel nanocomposite was successfully completed. The material was characterised and was found to be a suitable sensor for PAHs in water. An LOD of 0.19 µg·L^{-1} was obtained for phenanthrene under optimum conditions, while the LOD of anthracene, pyrene and naphthalene were estimated to be ~0.26 µg·L^{-1}. These results have been published in *Talanta* (Adegoke et al., 2015).

Overall, these first results regarding the novel fluorescence sensing of selected ECPs in water using QDs are very encouraging, and the proof of concept has been demonstrated successfully. The method shows remarkable sensitivity considering that the systems have not been fully optimised and no sample pretreatment or pre-concentration steps have been considered. Selectivity studies to determine the effects of potential interfering compounds are required and detection of the target ECPs in real water samples is planned in a follow-up study.

Experiments were undertaken where QD nanoparticles were immobilised into a silicone-based polymer namely PDMS. Thin film materials were fabricated on glass slides by means of spin-coating. These materials were fluorescent; however, their fluorescence was not optimal, hence an improvement of the immobilisation method is required. This may prove challenging, as access to a spin coater is limited and not all the items required for quality thin polymer film production are currently available. It is therefore envisaged that this component will be investigated in detail in a follow-up study.

Finally, an initial cost benefit analysis on using the QD fluorescence sensors was conducted. An evaluation of the costs that were incurred when synthesising the probes was done, and this was compared with costs of current conventional methods. The assessment shows that QD sensors may provide an economical alternative.

7 OVERALL CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

7.1 Overall Conclusions

ECPs are defined as new chemicals that do not have a regulatory status, but which may have an adverse effect on human health and the environment (Liu et al., 2014). Sources and environmental pathways of these ECPs have been increasingly associated with waste and waste waters arising from industrial, agricultural and municipal activities (Gavrilescu et al., 2014). The ECPs of current concern include a wide range of compounds including phthalates, PCBs, PAHs and bisphenol A, in addition to disinfectants, pharmaceuticals and hormones (Gavrilescu et al., 2014).

There is currently a lack of information regarding the nature, concentration, variability, transport and fate of ECPs in water, which is of global concern (Gavrilescu et al., 2014). In order to understand the potential biological impact of these chemicals, reliable and practical methods to identify and quantify ECPs in water are needed urgently. Analysis of ECPs is complex and challenging due to the wide range of compound classes involved, for which there are no established analytical methods in some cases (Liu et al., 2014). The sample matrix itself is often complex and may interfere with the target analyses and the ECPs are usually present at trace levels in water ($ng{\cdot}L^{-1}$ or $\mu g{\cdot}L^{-1}$).

The analysis of water for ECPs is of vital importance as many of these compounds are known to have adverse human health effects such as endocrine disruption, chronic toxicity, and the development of pathogen resistance to medication (Rosal et al., 2010), which makes a holistic understanding of exposure and risk necessary (Daughton, 2004). Data relating to the occurrence of ECPs in South African waters is currently very limited (Osunmakinde et al., 2013; Patterton, 2013), which can be ascribed in part to the high cost of performing the complex analyses involved. This project addresses this shortcoming by developing novel QD-based fluorescence sensors for target ECPs of relevance to South Africa. QDs have several optical properties, which make them ideal in this application, including high quantum yields, high molar extinction coefficients, broad absorption with narrow and symmetric PL spectra spanning the UV to the near-infrared, large effective Stokes shifts and high resistance to photobleaching (high photostability).

A **prioritisation process** was followed where a list of ECPs that had already been found to be priority pollutants as well as being known to be potentially hazardous in international studies was refined for it to be specifically applicable to South African circumstances and conditions. Usage or occurrence data as well as proxies for human and ecological health were employed in this process, which revealed the high priority which pesticides attained. GIS maps were generated based on the location of potential sources to identify vulnerable catchments and thereby to inform future ECP monitoring campaigns in the Gauteng Province.

For this proof of concept project, ECPs covering a range of compound classes were **selected as target analytes**. Specifically, compounds known to have environmental and human health concerns and/or are known to be used or produced in large quantities and are ubiquitous in the environment were selected, namely PAHs (as a compound class); atrazine (a pesticide); acetaminophen (a pharmaceutical) and triclosan (a PPC). PAHs are ubiquitous environmental pollutants, which arise from the combustion of organic fuels. They have potential human health effects including carcinogenicity and PAHs have been found in South African water systems at concentrations ranging from 0.1–52 $\mu g{\cdot}L^{-1}$ (Chimuka et al., 2015).

Organochlorine pesticides, like atrazine and terbuthylazine, are both widely used in agriculture in South Africa. These two pesticides have been identified as contaminants of emerging concern in South African water systems (Odendaal et al., 2015), hence they were be included as targets for fluorescence sensor development. The PPCPs have received much attention in environmental research. In spite of the fact that they exist at low concentrations, there is still a lot of concern regarding chemical persistence, microbial resistance, and synergistic effects of the numerous PPCPs present (Daughton & Ternes, 1999).

Triclosan is a synthetic, non-ionic, broad spectrum antimicrobial agent that has been used extensively for more than 20 years. It is a relatively stable, lipophilic compound that has been detected in sewage sludge, discharge effluent, receiving surface waters and sediments (Hua et al., 2005). Although triclosan is sparingly soluble in water (McAvoy et al., 2002) and waste water treatment processes can remove 58–99% of triclosan (NICNAS, 2009), environmental concentrations of 1.4–40 000 ng·L^{-1} have been found in surface water (SCCS, 2010). It is thus important to develop sensitive and selective methods to detect the environmental fate of triclosan. Acetaminophen is one of the most extensively used analgesic and antipyretic drugs, which can reduce fever and relieve moderate pain. It has been recognised as one of 95 contaminants in US streams (Kolpin et al., 2002). It is pervasive in the natural environment and it can easily accumulate in surface water, drinking water and waste water. Thus efficient, sensitive and simple analytical methodologies are of importance for the determination of acetaminophen which has been found at levels up to 10 000 ng·L^{-1} in surface water (Daughton & Ternes, 1999).

Water-soluble semiconductor CdSe/ZnS QDs capped with L-cys and NAC were **synthesised** by a one-pot process as a novel fluorescence sensor for the potential determination of triclosan and acetaminophen in water samples. Their structures were characterised by UV/Vis spectroscopy, fluorescence spectroscopy, FT-IR and powder XRD. The higher band-gap ZnS was directly grown onto monodisperse CdSe QD cores to protect the surface from the leakage of toxic cadmium ions into the solution in use. Furthermore, thiol ligands (L-cys and NAC) that were attached to the surface of ZnS not only provided water solubility but also may further prevent the leaching of cadmium ions. These QDs exhibited a remarkable stability which is attributed to the thiolate group anchoring to the surface of nanoparticles. GQDs were successfully prepared from oxidation of graphite as starting material. The GQDs had excellent optical properties, with high fluorescence intensity peaks depending on the excitation wavelength.

The synthesised water-soluble L-cys and GSH functionalised CdSe/ZnS QDs were used as **fluorescence sensors** to determine **acetaminophen** and **triclosan**, respectively. The operating mechanism of the designed probes was based on the fluorescence “turn-on” mode. Under optimum conditions with the addition of acetaminophen and/or triclosan to QDs, the original PL intensity of QD enhanced most likely due to fluorescence resonance energy transfer from analytes to QDs. The calibration plots were linear for both analytes and the methods could be applied to detect acetaminophen and triclosan at nanomolar levels. Other advantages of this method include simplicity, as well as stability and high resistivity of QDs against photobleaching (Dubertret et al., 2002).

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Experiments were undertaken where QD nanoparticles were **immobilised** into a silicone-based polymer, namely PDMS. Thin film materials were fabricated on glass slides by means of spin-coating. These materials were fluorescent; however, their fluorescence was not optimal, hence an improvement of the immobilisation method is required.

Finally, an initial **cost benefit analysis** on using the QD fluorescence sensors was conducted. An evaluation of the costs that were incurred when synthesising the probes was done, and this was compared with costs of current conventional methods. The assessment shows that QD sensors may provide an economical alternative.

7.2 Recommendations for Future Research

The creation of a universally accepted standard definition surrounding what exactly an ECP is and which compounds form part of this definition will greatly aid in the science of analysing and studying ECPs.

The literature review regarding occurrence information surrounding ECPs in South African water bodies has found that there is limited data available in this country. As such, the creation of a database that contains pertinent information surrounding the occurrence, toxicity (especially chronic toxicity), persistence and bioaccumulative potential of all ECPs would provide an invaluable resource from both a scientific and environmental point of view. This database should be in the public domain and scientists from across the country should be granted easy access it.

It is necessary that the technologies developed in this initial project be applied to the monitoring of the target ECPs in real water samples to optimise and validate the results and to determine effects of various variables and parameters (such as pH; contact time and interferences) on their performance. Enhanced selectivity via surface modification of the QDs should be investigated. In addition, further work is needed to optimise the immobilisation of the nanomaterials to enable reuse.

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APPENDIX 1: WARDS LOCATED ALONG VULNERABLE RIVERS AREAS

Table 25 lists all the wards located along the rivers inside the vulnerable areas that were identified during the GIS process. The people living in these wards may be at the highest risk of being exposed to ECPs if they use the water from the resource directly. Monitoring should be considered and measures to mitigate water contamination via ECPs should be implemented.

Table 25: Ward numbers of wards located along vulnerable rivers areas

Ward Number	Municipality
79800009	Johannesburg
79800010	Johannesburg
74202012	Meyerton
79700053	East Rand
79800013	Johannesburg
79800014	Johannesburg
79800023	Johannesburg
79800049	Johannesburg
79800050	Johannesburg
79800053	Johannesburg
79800071	Johannesburg
79800084	Johannesburg
79800119	Johannesburg
79800122	Johannesburg
79800127	Johannesburg
79800128	Johannesburg
79800130	Johannesburg
79700089	East Rand
79900007	Pretoria
79900048	Pretoria
79900057	Pretoria
79900061	Pretoria
79900065	Pretoria
79900066	Pretoria
79900069	Pretoria
79900070	Pretoria
79900078	Pretoria
79900091	Pretoria
4594	East Rand
4606	East Rand
4585	East Rand
4590	East Rand
4593	East Rand

APPENDIX 2: CAPACITY BUILDING AND KNOWLEDGE DISSEMINATION

Capacity Building

The students and their research topics listed below have contributed directly to the objectives of this project:

Hons research project report:

Miss Bianca Steytler (Graduated April 2016):

Novel analytical methods for atrazine pesticide determinations.

BSc Hons Chemistry, University of Pretoria.

Supervisor: Prof Patricia Forbes

MSc dissertation:

Mr Fritz Petersen (Graduated September 2016):

Prioritisation of emerging chemical pollutants in South African water resources.

MSc Water Resource Management, University of Pretoria.

Supervisor: Prof Patricia Forbes

Co-Supervisor: Dr James Dabrowski

PhD theses:

Mr Sifiso Nsibande (in progress):

Novel fluorescence sensors for pesticides and polycyclic aromatic hydrocarbons.

PhD Chemistry, University of Pretoria.

Supervisor: Prof Patricia Forbes

Miss Hanieh Montaseri (in progress):

Development of novel fluorescence sensors for emerging chemical contaminants.

PhD Chemistry, University of Pretoria.

Supervisor: Prof Patricia Forbes

Knowledge Dissemination

Published journal papers:

Oluwasesan Adegoke and Patricia B.C. Forbes, L-cysteine-capped core/shell/shell quantum dot-graphene oxide nanocomposite fluorescence probe for polycyclic aromatic hydrocarbon detection, *Talanta*, 2016, 146, 780-788.

Hanieh Montaseri and Patricia Forbes, A review of monitoring methods for triclosan and its occurrence in aquatic environments, *TrAC Trends in Analytical Chemistry*, 2016, 85, 221-231.

Sifiso Nsibande and Patricia Forbes, Fluorescence detection of pesticides using quantum dot materials – A review, *Analytica Chimica Acta,* 2016, 945, 9-22.

Fritz Petersen, James Dabrowski and Patricia Forbes, Identifying potential emerging chemical pollutant surface water sampling sites in the Gauteng Province, South Africa, *Water SA,* 2017, 43(1), 153-165.

Journal paper accepted for publication:

Oluwasesan Adegoke, Tebello Nyokong, Patricia B.C. Forbes, Photophysical properties of a series of alloyed and non-alloyed water-soluble L-cysteine-capped core quantum dots, *Journal of Alloys and Compounds* in press.

Conference presentations:

Fritz Petersen, James Dabrowski and Patricia Forbes, "Prioritisation of Emerging Chemical Pollutants in South African Water Systems", Water Institute of South Africa 4th Young Water Professionals ZA Biennial and 1st African Young Water Professionals Conference, 16-18 November 2015, CSIR Convention Centre, Pretoria.

Plenary lecture: Patricia Forbes, "Novel approaches to environmental monitoring", International Conference on Pure and Applied Chemistry 2016, 18-22 July 2016, Mauritius.