# Scoping study and research strategy development on currently known and emerging contaminants influencing drinking water quality

Report to the
**Water Research Commission**

by

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This report contains a CD with some of the data measured in this project.

# Reference Group

| | |
|---|---|
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# List of Abbreviations

| | |
|---|---|
| µg | microgram ($10^{-6}$ g) |
| AIDS | Acquired Immuno-Deficiency Syndrome |
| ATR | Atrazine |
| ATSDR | Agency for Toxic Substances and Disease Registry |
| BET | Best Available Technique |
| CASRN | Chemical Abstract Services Registry Number |
| CBZ | Carbamazepine |
| CCL | Contaminant Candidate list |
| CEN | Comité Européen de Normalisation (European Committee for Standardization) |
| Cv | Coefficient of variation |
| DALY | Disability-Adjusted Life Years |
| DBP | Disinfectant By-products |
| $ddH_2O$ | Doubly distilled water |
| DDL | Data Definition Language |
| DDW | De-ionised distilled water |
| DHHS | Department of Health and Human Services (of the United States) |
| DML | Data Manipulation Language |
| DWD | Drinking Water Directive |
| EC | Emerging Contaminant |
| ECD | Electron Capture Detection |
| ECs | Emerging contaminants |
| EPA | Environmental Protection Agency |
| ESI-LC-MS | Electron spray ionisation liquid chromatography mass spectrometry |
| ETBE | Ethyl Tertiary-Butyl Ether |
| EU | European Union |
| FA | Formic Acid |
| FD | Fluorescence Detection |
| FDA | Food and Drug Administration |
| FID | Flame Ionisation Detection |
| g | gram |
| GC | Gas Chromatography |
| GV | Guideline Value |
| HPLC | High performance liquid chromatography |
| HRL | Health Reference Level |
| IARC | International Agency for Research on Cancer |
| IRIS | Integrated Risk Information System |
| IS | Internal standard |
| ISO | International Organisation for Standardisation |
| IUGR | Intra Uterine Growth Retardation |
| kg | kilogram ($10^3$ g) |
| L | litre |

| | |
|---|---|
| LC | Liquid chromatography |
| LC-MS-MS | Liquid Chromatography Tandem Mass Spectrometry |
| LD50 | Dose Lethal to 50% of a Population |
| LLOQ | Lower limit of quantification |
| LOD | Limit Of Detection |
| MAC | Maximum Allowed Concentrations |
| MBTE | Methyl Tertiary-Butyl Ether |
| MCL | Maximum Contaminant Levels |
| MCLG | Maximum Contaminant Level Goal |
| MeOH | Methanol |
| mg | milligram ($10^{-3}$ g) |
| mL | millilitre ($10^{-3}$ L) |
| MS | Mass Spectrometry |
| NAS | National Academy of Sciences |
| NDWAC | National Drinking Water Advisory Council |
| ng | nanogram ($10^{-9}$ g) |
| NIEHS | National Institute for Environmental and Health Sciences |
| NIH | National Institutes of Health |
| NOAEL | No-Observed-Adverse-Effect Level |
| OPP | Office of Pesticide Programs |
| PCCL | Preliminary Contaminant Candidate List |
| PCP | Personal Care Products |
| PCR | Polymerase Chain Reaction |
| ppb | Parts per billion |
| ppm | Parts per million |
| ppt | Parts per trillion |
| PSI | Pounds per square inch |
| QA | Quality Assurance |
| QC | Quality Control |
| QMRA | Quantitative Microbial Risk Assessment |
| RfD | Reference Dose |
| RP | Reverse phase |
| S/N | Signal-to-noise ratio |
| SDWA | Safe Drinking Water Act |
| SPE | Solid phase extraction |
| TBA | Terbuthylazine |
| TDI | Tolerated Daily Intake |
| TT | Treatment Technique |
| TWAC | Time-weighted average concentration |
| U-HPLC-MS | Ultra high performance liquid chromatography mass spectrometry |
| ULOQ | Upper limit of quantification |
| USGS | United States Geological Survey |
| UV | Ultra Violet |
| WHO | World Health Organisation |

| | |
|---|---|
| WRC | Water Research Commission |
| WSP | Water Safety Plan |

# 1 Executive Summary

The aim of this study was to investigate and identify the most important new substances in drinking water that could be a concern to human health in South Africa.

The specific aims were:

1. Complete a comprehensive review of literature on emerging contaminants (ECs)
2. Identification of three most critical ECs in South Africa
3. Review of current methods to analyse and quantitate ECs in water
4. Complete a national reconnaissance study on the three critical ECs
5. Development of risk matrix for the three critical ECs
6. Define critical issues that must be addressed regarding ECs
7. Identify knowledge and skill gaps, propose a future research strategy and develop a Terms of Reference for the research

The rapid advances made in improving the sensitivity of analytical equipment and methodologies have allowed the detection of chemical compounds and microorganisms at exceedingly low levels in drinking water. This has contributed to the detection of an expanding list of compounds that were previously not observed, and has raised a general concern about contaminants for which no regulatory guidelines exist. These are collectively referred to as emerging contaminants (ECs), and include the partially over-lapping groups of pharmaceuticals, pesticides, endocrine disrupting compounds, cyanotoxins, personal care products, industrial and manufacturing chemicals and microorganisms, including bacteria, viruses, and parasites such as helminths and protozoa.

A comprehensive review of the screening programmes or activities of the World Health Organisation (WHO), Environmental Protection Agency (EPA), European Union Commission, Unites States Geological Survey (USGS) and the National Institute of Environmental Health Sciences aimed at addressing concerns related to these ECs, was undertaken. We found that primarily the WHO, EPA and USGS had programmes to identify ECs, and facilitated research to generate scientific data on the possible health risk represented by these ECs. This, together with the environmental concentration of an EC, and the size of a potential exposed population contributed to decisions on whether the level of a given EC in drinking water should be regulated.

Since chemicals and microorganisms that are present in drinking water in the EU or the US may not necessarily be present in drinking water in South Africa, as well as the fact that some chemicals for which guideline values exist may still be unregulated in South Africa, we performed a limited, qualitative survey on drinking water sampled on multiple occasions in two major cities in South Africa. We concentrated on the detection of polar, water soluble compounds. This provided a significantly smaller list of contaminants that may be present in SA drinking water. A careful consideration of the severity of the possible health effects of each of the identified contaminants finally provided three chemical determinants with the highest potential of having a negative health impact. These were the herbicides atrazine and terbuthylazine, and the anticonvulsant, carbamazepine. We therefore undertook an extensive national survey on the concentration

of these three chemicals in drinking water of several metropolitan areas. A qualitative screening was also performed on each of the water samples.

A review of the literature showed than liquid chromatography linked to tandem mass spectrometry (LC-MS/MS) was a modern technique routinely applied to the detection and quantitation of polar, water soluble compounds including pesticides and pharmaceuticals. This technique is capable of detecting quantities in the ng/L range, with the exact lower limit depending on the instrument configuration. LC-MS/MS has also been successfully applied in the past to the quantitation of atrazine, carbamazepine and terbuthylazine. For these reasons we decided to develop and validate an LC-MS/MS quantitation method for this study.

Food and Drug Administration (FDA) guidelines of method validation was followed. According to FDA guidelines, a valid method must meet specific criteria in terms of accuracy, precision, selectivity, sensitivity, reproducibility and stability. The method must be able to quantitate a compound in a mixture of compounds, determine the concentration within 15% of the real value, and replicate quantitated levels. Furthermore, the method must be able to reproducibly recover > 40% of a compound from a complex mixture, and the compound of interest as well as quantitation standards must be stable during the manipulations associated with the quantitation method.

During method development we rigorously evaluated the conformance of the method in terms of each of these criteria. We specifically established, routinely using triplicate determinations, that our method was selective for the compound of interest, and that there was no detectable interference between the three compounds during either extraction or quantitation. Dilutions were used to establish the lower limit of quantitation (LLOQ) as well as the upper limit of quantitation (ULOQ). The LLOQ was defined as the concentration where the signal-to-noise ratio exceeded the value of 10, and was determined at 50 ng/L for carbamazepine and terbuthylazine, and at 100 ng/L for atrazine. The ULOQ was determined at 100 µg/L for each of the three compounds, based on the minimum dilution where the calibration curve still exhibited approximate linear behaviour. Compound stability was also verified by triplicate quantitation over a 24h period of diluted and stock concentrations of each of the three standards.

Samples were collected from water purification plants in Bloemfontein, Johannesburg, Pretoria, Durban, Pietermaritzburg, Port Elizabeth and Cape Town at points before the water entered the reticulation system. An agreement that was reached with some water purification plants dictated that the water purification plants could not be identified beyond the metropolitan city that is served. Water was also collected from domestic taps in southern and northern Bloemfontein, served by different water sources. The collections were done in February, May, August and November of 2012. The samples were returned to the laboratory for processing and quantitation within 48h after collection.

Water was passed through Strata 18 CE 200 mg solid phase extraction cartridges (SPE), which gave the best recovery of all SPEs tested. Samples were eluted, and quantitated by LC-MS/MS on a high-performance liquid chromatography system linked to a QTRAP 3200 mass spectrometer. Quantitation was carried out by multiple reaction monitoring, using published precursor and fragment masses.

A combined total of 34 pharmaceuticals and pesticides from 618 tested, were detected in the water samples over a 4 season period. In line with the preliminary screen, atrazine, carbamazepine and terbuthylazine were detected in the highest number of water samples and with the most number of seasonal occurrences. Apart from these compounds, compounds that were detected in 3 or more seasons included hexazinone, phenytoin, and tebuthiuron (Durban), tebuthiuron (Johannesburg), and fluconazole, phenytoin and tebuthiuron (Bloemfontein). The antimalarial, cinchonidine, was detected in at least 3 season in each of the seven cities that formed part of this study.

Quantitation of the herbicide atrazine showed that it was present at elevated levels (approximately 12 ng/L) compared to the other cities, in each of the four seasons in Johannesburg. A similar elevated seasonal presence was observed in Johannesburg for the herbicide terbuthylazine, which was present at approximately 12 ng/L in each season. The anticonvulsant and mood-stabilising drug, carbamazepine, was present at elevated levels (approximately 200 ng/L) in all four seasons in Bloemfontein. The highest level of atrazine (163 ng/L) and terbuthylazine (206 ng/L) determined, were in Pretoria in the autumn. The highest level of carbamazepine was 324 ng/L in Bloemfontein in the summer. The maximum contaminant level, a level set by the EPA to be as close as is economically feasible to the maximum contaminant level goal, a level where there is no known or expected risk to health, was stipulated at 3 µg/L for atrazine and terbuthylazine, and 12 µg/L for carbamazepine. Thus, the maximum levels detected for each of the three surveyed compounds never exceeded the stipulated MCL. In fact, the maximum never exceeded 6% and 7% of the MCL in the case of atrazine and terbuthylazine, respectively. In the case of carbamazepine, the highest detected level did not exceed 3% of the MCL. It therefore appears that even the highest recorded levels of the three ECs included in this survey never approached a level where it would be expected to have an impact on human health.

A major consideration when determining the observable impact of contaminants in drinking water is not only the quantitated level of the contaminant, but also the size and demographics of the population that would routinely consume the water. This impact is generally expressed as a risk and is a function of the hazard, the vulnerability of the population, and the capacity of the population to overcome the hazard. The severity of a hazard can be expressed on a scale from 1 to 5, corresponding to levels where the effect of the hazard is described as negligible and increasing to a level where it is described as extremely severe. In order to map the hazard severity level of a compound, detailed data on the health impact, preferably from medical case studies or epidemiological studies, must be available, including exposure concentrations, conditions, and health effects. Very little precise data is available over an environmentally relevant concentration range in the case of atrazine, terbuthylazine and carbamazepine. However, by making use of available data, we were able, as a first approximation, to roughly correlate the concentrations of these compounds to severity levels. A hazard risk matrix was then developed by combining the severity of the hazard with the frequency of its occurrence. This allowed us to propose a risk matrix ranging from 1 to 20, representing the range from low severity/low frequency to high severity/high frequency risks. Since the average as well as the highest quantitated levels of the three tested ECs were well below the MCL, we proposed a severity score of 1 for each. The hazard risk was then mostly an effect of the fractional seasonal occurrence of each of the tested ECs. Using banded ranges, the occurrence of atrazine and terbuthylazine in the water sourced from Bloemfontein, Johannesburg, Durban and Pietermaritzburg was found to represent a medium hazard. Terbuthylazine was a medium risk in the water from Cape Town. Carbamazepine was found to be a medium

risk in the water from Johannesburg, Pretoria, Bloemfontein, Durban and Pietermaritzburg, Cape Town and Port Elizabeth. However, the frequency and level of the detected compounds did not require any specific and aggressive remedial action.

We note that careful attention must be given to what is understood by risk, hazard and health impact, and these concepts must be used consistently. It is also necessary to be extremely rigorous to arrive at an accurate risk assessment, and any assumptions made in that assessment must be clearly stated.

This study was very valuable in that it demonstrated the presence of a range of emerging contaminants in South African drinking water. Several areas were also clearly identified that require further research to fully understand the possible impact of ECs on the South African water consumer. Although the quantitated levels of the three most frequently observed ECs were less than 10% of their respective MCLs, the range of EC observed may indicate a growing problem. A national programme in which drinking water is seasonally or bi-annually qualitatively screened, and frequently observed ECs quantitated, should be considered. Furthermore, the proposed hazard risk matrix showed than we lacked information on the vulnerability of populations and their capacity to overcome the posed hazard. This ability is particularly acute for economically repressed, rural population that were excluded by the scope of this study. It is recommended that a similar qualitative screen and quantitation of the level of select, identified ECs be undertaken in one or more rural communities that routinely use raw water directly from rivers or dams. Lastly, medical waste and pesticides are often dumped in unprepared locations, where leaching of pharmaceuticals and pesticides into groundwater reservoirs is possible. The contamination of groundwater, and retrieval and use of such water through boreholes, remain unexplored. We suggest a study on the presence of pharmaceuticals in borehole water due to leaching from medical waste dumping grounds.

# 2 Introduction

## 2.1 Background

Over the past decade studies on contaminant levels in European and North American drinking water revealed the presence of an extensive range of extraneous chemicals, including pesticides, pharmaceuticals, industrial and manufacturing chemicals and personal care products. In fact, in a study published in 2002, the US Geological Survey reported (Kolpin et al., 2002; Kolpin et al., 1998) that pharmaceutical compounds, hormones or organic wastewater contaminants were detectable in 80% of all surface water sources sampled in the USA. These contaminants included steroids, analgesics, mood disorder and heart medication, as well as many other classes of compounds. Some of these compounds were known carcinogens or endocrine disruptors, and may pose a severe health risk to humans and the environment. The majority of these chemicals as well as emergent pathogenic microorganisms are not legislatively regulated, and their levels, seasonal fluctuations and effects on health at environmentally observed concentrations are unknown. These chemicals are collectively referred to as "emerging contaminants" (ECs), and can be divided into the following (partially overlapping) groups:

- Pharmaceuticals
- Pesticides (herbicides, insecticides, fungicides, etc.)
- Hormones
- Endocrine disruptors
- Disinfection by products (DBPs)
- Personal care products (PCPs)
- Industrial and manufacturing chemicals
- Organic solvents MTBE and ETBE
- Recreational and non-controlled drugs

Advances in mass spectrometry and refinement of the polymerase chain reaction have allowed the highly sensitive detection and identification of ECs in water samples, thus increasingly attracting the attention of water quality administrators and environmental scientists. To date no comprehensive, national survey has been undertaken on the presence of ECs in drinking water in South Africa. This is of great concern to water health regulators considering the findings from Europe and the US.

It is therefore crucial to significantly expand our knowledge on ECs that may be present in South African drinking water, and to develop a coherent scientific response to this presence. This Water Research Commission project represents part of such a focussed response.

## 2.2 Aims

The specific aims that were addressed in his study were:

1. Complete a comprehensive review of literature on emerging contaminants (ECs)
2. Identification of three most critical ECs in South Africa
3. Review of current methods to analyse and quantitate ECs in water
4. Complete a national reconnaissance study on the three critical ECs
5. Development of risk matrix for the three critical ECs
6. Define critical issues that must be address regarding ECs
7. Identify knowledge and skill gaps, propose a future research strategy and develop a Terms of Reference for the research

# 3 Overview of Management of ECs in Drinking Water Globally

ECs are a collection of environmental chemicals that were either formerly not deemed as hazardous or that were not known to occur in the environment, but now seem increasingly suspect due to improved detection methods, and with current toxicological data. These chemicals are not routinely monitored, and have no allowed maximum limit for specific environments. Knowledge about an EC's source, toxicity, bio-accumulation, occurrence, transport and transformation and degradation mechanisms are crucial to evaluate its possible health risk.

ECs are a global problem. The detection and regulation of these contaminants in drinking water demand a concerted world-wide effort. The next sections will discuss the approaches that organisations from around the world have taken to determine *which* contaminants should be regulated, and *how* these contaminants should be regulated.

## 3.1 World Health Organisation (WHO)

The establishment of a global health organisation had been envisioned by the United Nations since its founding in 1945. Three years later saw the inception of the World Health Organisation (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB; Who, 2011) which has since acted as the authority responsible for directing and coordinating health based issues at the United Nations. Consequently, WHO advises and collaborates with many environmental agencies and groupings to protect, amongst other things, the quality of drinking water. The WHO also aids the agencies by establishing guidelines and providing advice about the classification of water-borne entities of health concern, including microbial agents and chemicals.

WHO regularly releases guidelines for drinking water quality. These guidelines aid many drinking water authorities with the establishment of frameworks to monitor and improve water quality. The current edition (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB; Who, 2011) was released in June 2011, and is discussed below.

### 3.1.1 Proposed framework for safe drinking water

In order to discuss the method that is used by the WHO to define an EC, it is necessary to give the background to the system that is used by the WHO to manage water safety. The foundation for ensuring safe drinking water is the development of a Water Safety Plan (WSP) based on a Framework for Safe Drinking Water (see **Error! Reference source not found.**). This framework must be aligned with the guidelines provided by the WHO, and should offer a preventative, risk-based approach to manage water quality. Firstly, the framework should include health-based targets based on the guidelines, and these should be accepted by an experienced health authority taking cognisance of specific local environmental, societal, population and health issues. Secondly, WSPs should be put in place allowing assessment of water quality from source to consumer. It should also allow the operational monitoring of the control measures in the supply chain, the documentation of assessment and monitoring activities according to a management

plan, and stipulate specific actions under defined conditions. The WSPs should be independently verified, and compliance of the whole process with the stipulated health based targets must be confirmed.

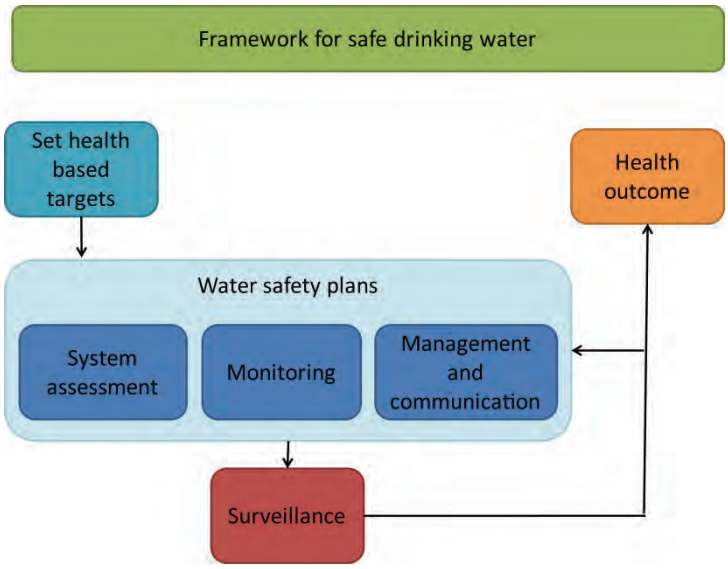


**Figure 1. Framework for safe drinking water (adapted from WHO 2011)**

#### *3.1.1.1 Health based targets*

Health-based targets are a necessary part of the drinking water safety framework (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB; Who, 2011). These targets are generally set by an experienced, high-level authority, which should include professionals from water suppliers as well as affected communities. Health targets should consider the general public health and the role of drinking water quality in ensuring public health.

Health-based targets form the foundation of ensuring the supply of good quality water to consumers. When characterising health effects, it is important to distinguish between compounds that cause adverse health effects from single exposures (e.g. pathogenic microorganisms), and compounds that cause adverse effects due to a persistent, longer period of exposure (e.g. chemicals). Due to different attributes such as environmental factors in water, contaminant mechanisms of action and fluctuations in concentrations, there are four main classes of health targets. These classes are classified according to safety requirements. These targets are:

- **Health outcome targets**
  Health outcome targets are the most direct measure of drinking water quality, and specify upper limits to the acceptable frequencies of diseases such as diarrhoea or cancer. The acceptable disease burden is generally set at a national level. Although such numeric representations are required for

the development of quantifiable water safety frameworks, the acceptable disease burden does not take the severity of a disease into account. The WHO therefore employs a metric known as the Disability-Adjusted Life Years (DALY), set at $10^{-6}$, to represent a disease burden. A DALY of $10^{-6}$ per person per year is approximately equivalent to a $10^{-5}$ excess lifetime risk of developing a disease, i.e., the occurrence of one incident of a disease due to consumption of water over a 70 year period in a population group of 100,000 individuals (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB; Who, 2011). In the case of "threshold chemicals", or chemicals that have a clear toxic effect when exposure exceeds a specific level, a no-observed-adverse-effect level (NOAEL) is usually set. For "non-threshold chemicals" such as genotoxic carcinogens, a NOAEL of 0 is usually specified.

- **Water quality targets**
  Water quality targets are widely used as a health-based criterion to set acceptable levels for chemicals found in water. These levels are generally specified based on the expected risk of developing health effects associated with the consumption of water.
- **Performance targets**
  Although performance targets can be set to specify chemical levels, they are more generally applied to manage microbial and viral contamination of drinking water. Performance targets typically express a target level for a water-borne pathogen relative to the level in the water source, and are based on health outcome targets of a tolerable disease burden. Also, since it is generally impractical to routinely test for a wide range of pathogens, indicator microorganisms that are representative members from the bacterial, viral and protozoan groups are used, and performance targets are typically set at log reduction. For instance, *Escherichia coli* is generally used as an indicator of recent faecal contamination of drinking water. Performance targets are generally set when constituents pose adverse health effects due to a single exposure or when large fluctuations in concentration occur over short periods with significant implications for health.
- **Specified technology targets**
  Water protection authorities may set specified technology targets for smaller municipalities and communities. These targets are usually in response to local water quality issues, and may include recommendations to include filtration and disinfection of surface water to meet health outcome targets.

#### *3.1.1.2 Water safety plans*

The development of water safety plans is crucial for the successful regulation of contaminant levels in drinking-water. A water safety plan is typically composed of system assessment, operational monitoring, and management modules. These modules ensure a decrease in contaminant concentration, while also reducing public health risks. Water safety plans are developed according to the principle of multiple barriers, hazard analysis and critical control points and other systematic management approaches. Such plans address all aspects of drinking water sources and focus on examining and treating contaminated drinking water. In general, water safety plans involve the suitable documentation of systematic assessment and prioritisation of hazards, which includes limiting contaminants.

#### *3.1.1.3 Surveillance*

The last component of the framework includes the surveillance of the whole framework via an external organisation. This organisation has the authority to revise and improve the current health-based targets with regard to health outcomes.

### 3.1.2 Developing priority lists of chemicals that require guidelines

Many of the chemicals that are listed in the WHO guidelines are not present in drinking water everywhere. Conversely, there may be chemicals present at some localities for which there are no WHO guidelines. It is not practical to perform a comprehensive screening on a continual basis to try and detect all chemicals or microorganisms present in drinking water. In fact, such an approach may limit the availability of scarce resources that may be more meaningfully applied to other aspects of drinking water quality management. Thus, when deciding on the routine screening of a chemical, two important points must be considered:

- The probability that consumers are exposed to this chemical in drinking water must be high
- Exposure to the chemical must represent a significant health hazard

When deciding on consumer exposure, attention should also be given to alternative routes of exposure. For instance, if consumers are exposed to a significant level of an air-borne contaminant or pesticide residue in food, it is unlikely that limiting exposure to a low level in drinking water will have a measurable and positive impact on public health. In cases of a clear increase in the occurrence of a disease within a confined geographic locality, for example arsenicosis, it is reasonable to analyse drinking water for the causative chemical. However, where national and local disease surveillance and epidemiological data is lacking, it is necessary to consider the likely exposure of humans to a chemical, and the health impact of that exposure. This can involve the comprehensive desktop review of relevant toxicology literature.

It is also important to consider the potential source of the chemical as well as its transport routes to drinking water. Thus, in practical terms, if it is known that upstream industrial pollution may contaminate drinking water, appropriate chemicals to consider would be those expected at the point of pollution. Generally, this would involve knowledge of chemicals that would be produced by the relevant industrial processes, as well as confirmation that these chemicals are introduced in upstream rivers, catchment areas, or dams. In some instances, environmental disasters, such as the accidental spillage of a chemical, directly defines the chemical that should be screened for in drinking water, provided the transport route is likely to lead to the introduction of the chemical into drinking water, and toxicological data or an established guideline value clearly indicates a health risk.

Thus, when considering the screening of ECs, the WHO recommends a review of toxicological and epidemiological data, where available, as well as a consideration of the likelihood that the specific chemical will be present in drinking water at a level that represents a health risk. Where a chemical of concern may be present at levels that could periodically exceed the NOAEL, it should be included in a list that is routinely

monitored. However, this will require a survey of the defined chemicals of concern to follow the seasonal variation in levels linked to seasonal agricultural activities or to rainfall.

### 3.1.3 Defining the health impact of a chemical or microorganism

When considering toxicity of a "threshold chemical", there is a dose below which no adverse effect is thought to occur. This is known as the Tolerated Daily Intake (TDI), and is defined as the NOAEL adjusted for uncertainty (due to inter-species or intra-species variation, dataset quality or health effect severity) or by a factor related to the specific chemical (based on the expert review of quantitative toxicokinetic and toxicodynamic data), and is expressed as µg or mg/kg bodyweight. The Guideline Value (GV) is derived from the TDI as represented by the following equation:

$$GV = \frac{TDI \times bw \times P}{C} \qquad \text{(Eq. 1)}$$

where,

*bw* is the bodyweight in kilograms
*P* is the fraction of the TDI allocated to drinking water
*C* is the daily consumption of drinking water in litres

The WHO has published extensive lists of GV values for a range of chemicals in drinking water (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB; Who, 2011).

In the case of "non-threshold chemicals" or genotoxic carcinogens, the compound introduces a mutation in the DNA of a somatic cell. The risk of this mutation is present irrespective of the level of the compound, hence the terminology "non-threshold chemical". When deriving GV values for carcinogens, consideration is given to the mechanism whereby the chemical exerts its effect. This generally involves long-term laboratory animal studies, or, in the case of humans, occupational exposure. This data is used by a division of the WHO, the International Agency for Research on Cancer (IARC) that classifies compounds by carcinogenic risk assessment as well as qualitative risk assessment. The GV values of non-threshold chemicals by the WHO are based on such IARC assessments.

Drinking water is generally monitored for the presence of indicator organisms such as *E. coli*, where the presence of this bacterium implies recent faecal contamination. There are many other bacteria, viruses and protozoa that have been identified in drinking water, and that generally do not pose a health risk. However, with climate change, population growth, human movement and the increasing impact of human activities on the environment, new microbes with health concerns do arise. Pathogenic strains may re-emerge or the transfer of infectious agents from animals to humans, known as zoonoses, may occur. The WHO reported that between 1972 and 1999, 35 new disease agents had been discovered, many of them transmittable by water (Ellenhorn et al., 1997a; Who, 2003). These include *Cryptosporidium*, *Legionella*, *Escherichia coli* O157 (E. coli O157), rotavirus, hepatitis E virus and norovirus (Bingham et al., 2001a; Sherman et al.). It is

estimated that worldwide 26% of deaths are caused by infectious diseases, many of them water-borne. It should therefore be an integral part of a safe water framework to consider the presence of emergent pathogenic organisms in drinking water.

However, as with emerging chemicals, it is not a tractable exercise to screen for extensive lists of possible pathogens. A mathematical framework known as Quantitative Microbial Risk Assessment (QMRA) can be used to evaluate the risk of infection by human pathogens, as well as managing waterborne microbial hazards, especially sporadic hazards. QMRA is generally used to calculate acceptable disease burdens, and is based on an assessment of exposure, i.e. to the number and identity of the pathogen to which a population is exposed, as well as the dose-response, i.e., the probability of an adverse health effect following exposure. The most frequently applied health based targeting to manage water-borne microorganisms is performance targeting.

The WHO suggests that the choice of an emerging pathogen should be based on local epidemiological or disease epidemic data, as well as local population and socioeconomic factors, such as the presence of sub-populations of immuno-compromised individuals.

A list of ECs as defined by the WHO is presented in Appendix A.

## 3.2 Environmental Protection Agency (EPA)

The EPA was founded in 1970 when environmental pollution and its effects on ecology became an increasing concern in the United States (US). The primary purpose of the EPA is to have a single organisation doing a range of federal research aimed at ensuring a healthy environment. This protection is accomplished by monitoring, the setting of standards, and enforcement (Jackson, 2011; Waller et al., 2010).

Many laws permit the EPA to ensure and protect the quality of water in the US. The US federal law that ensures the quality of drinking water is the Safe Drinking Water Act (SDWA). According to the SDWA, the EPA must periodically release a Contaminant Candidate List (CCL). A CCL is a source list that contains priority contaminants. Research is done on the entries on the CCL to decide which regulatory techniques should be implemented to manage the presence of a contaminant in drinking water.

To date three CCLs have been published by the EPA. The latest CCL (CCL3) was released in August 2009. The first two CCLs were based on readily available information on about 50 chemicals and 10 microbial contaminants that were reviewed by technical experts. The development of CCL1 was mediated by consultations with the scientific community and the National Drinking Water Advisory Council (NDWAC). CCL2 contained contaminants from CCL1 that were not removed after the first regulatory determination processes.

Input from the National Academy of Sciences (NAS) on the methodologies of assembling the first two CCLs, led to the development of new methods to construct the latest CCL. Hence, CCL3 was developed to focus on

possible ECs as well as existing contaminants. A preliminary contaminant candidate list (PCCL) was first constructed and then refined. After a detailed assessment using a classification approach together with expert judgement, the likelihood that specific contaminants occurred in drinking water at levels that posed a public health risk was determined, producing CCL3 (see Appendix B).

### 3.2.1 EPA's responsibilities under the SDWA

The SDWA obliged the EPA to publish a Maximum Contaminant Level Goal (MCLG) and to propagate reasonable benchmarks to water suppliers if a contaminant satisfied the following 3 criteria:

- Contaminant have an adverse effect on human health
- Contaminant is known to exist in or are likely to occur in public water systems at concentrations of concern
- Regulation of a contaminant will produce a significant decrease in health risk, according to the judgement of the Administrator

Contaminants that satisfy these criteria require regulation. According to legislation, the EPA has a maximum period of 24 months in which to publish a proposed MCLG and corresponding regulatory standards. After this initial period, Maximum Contaminant Levels (MCLs) and standards must be established within a further 18 months.

### 3.2.2 Determining candidate contaminants

The most important consideration when identifying candidates is the availability of adequate information. This information should include data on potential health effects and known or likely exposure via drinking water. During the determination of potential health effects, the EPA first inspects agency-approved assessment information for sources, like the Integrated Risk Information System (IRIS), the Agency's Office of Pesticide Programs (OPP), the National Academy of Sciences (NAS) and the Agency for Toxic Substances and Disease Registry (ATSDR). The determination of occurrence is informed by the collection of occurrence data that realistically represents actual or likely occurrence of contaminants in public water systems. In addition to this information, any supplementary data about appropriate analytical and treatment methods are gathered. If this information is available, the EPA defines the contaminant as a potential candidate for regulatory determinations.

Regulations are introduced based on the criteria below.

#### *3.2.2.1 Does the contaminant pose a health risk to consumers?*

This is determined using the best available, peer-reviewed assessments and studies to characterise the human health effects that may result from exposure via drinking water. According to this information, a health reference level (HRL) is established.

#### *3.2.2.2 Is the contaminant likely to occur at a concentration of concern?*

This information is gathered by screening occurrence datasets. The decision on which contaminants to screen and analyse depends on the HRLs determined earlier. The HRL estimate is used as a threshold to identify PWS that supplied water to an estimated number of consumers and that had occurrence levels (concentrations) higher than half of the HRL. The occurrences of these contaminants are further aided by analysing information on the use and release of the contaminants into drinking water. The EPA also uses supplemental information on occurrence in water from studies performed by other agencies, organisations and/or entities such as the United States Geological Survey.

#### *3.2.2.3 Meaningful opportunity for health risk reduction?*

The integration of information about potential health effects and the estimates of occurrence, as well as estimates of exposure at levels of health concern, informs the administrator in his/her determination on the need and adequacy of regulations.

If a contaminant conforms to all three criteria, the contaminant is included in the EPA's candidate contaminant list (CCL), which means that the contaminant requires regulation.

Monitoring of a contaminant determined to require regulation starts with research which enables the EPA to make an informed decision on the setting of an MCLG. The MCLG stipulates the lowest level (within a safety margin) at which a contaminant must be present in drinking water to have an adverse effect on human health. During the determination of MCLGs, the risk of exposure to sensitive sub-populations (infants, pregnant women, children and the elderly) is also considered. Accordingly, if a microbial contaminant poses a health risk, MCLG is set to zero, since ingestion of such a contaminant may result in adverse effects. Secondly, if a carcinogenic contaminant has no suggested safe dose for ingestion, the level of the carcinogenic contaminants is also set to zero. Finally, if chemicals are known to have a non-carcinogenic adverse health effect, the MCLG is based on a reference dose (RfD), which is an estimate of the anticipated amount of chemical that a person could regularly be exposed to throughout his/her lifetime, without developing any adverse effects.

MCLGs only take into account public health goals, and are therefore not always complied with. Issues such as possible limitations in detection and treatment should also be considered. Consequently, after MCLGs are determined, the EPA establishes a set of enforceable standards. These enforceable standards are called the Maximum Contaminant Level (MCL), which is the maximum allowable level of a contaminant in water which is delivered to any user of the public water system. When detection of contaminants at extremely low levels is economically or technically possible, a Treatment Technique (TT) rather than an MCL is enforced. This TT should be followed to ensure contaminant control. Common examples of such treatments are the Surface Water Treatment Rule (http://water.epa.gov/lawsregs/ rulesregs/sdwa/mdbp/lt1/lt1eswtr.cfm) and Lead and Copper Rule (http://water.epa.gov/lawsregs/rulesregs/sdwa/lcr/index.cfm).

### 3.2.3 Components of risk assessment

The EPA makes use of an integrated database, IRIS, which contains information on human health effects due to exposure to various chemicals. The database contains more than 550 chemical substances. If no information on a substance is available in IRIS, the EPA analyses risk factors in-house. The EPA defines risk as the probability that an EC can cause injury, disease or death after exposure, either alone or in combination with other chemicals. The process of establishing regulatory rules for an EC is preceded by a risk assessment. A risk assessment can be subdivided into the steps shown in Figure 2.

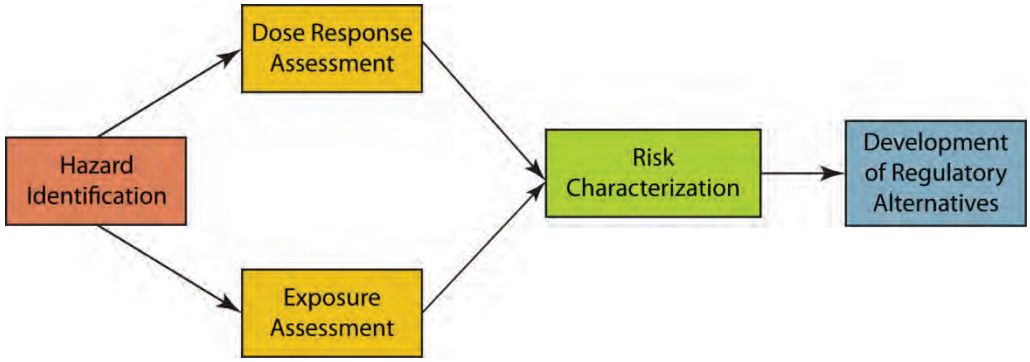


**Figure 2. Steps of risk assessment of ECs**

The first step towards risk assessment is identifying a link between a contaminant and a specific health hazard based on research on animals or other test organisms. This is followed by determining the amount of a contaminant that leads to adverse health effects, as well as the time of exposure, contaminant concentration and population size that is exposed to the contaminant. These three categories are then integrated to characterise the consequences of the risk. Typically, risks are categorised according to adverse health effects caused by different forms of exposure. The EPA defines these classes as hazardous effects due to acute exposure, chronic exposure and exposure during critical periods. The final step in assessment of the risk is to identify potential regulatory methods and assess their effects to determine the most efficient method of regulation.

#### *3.2.3.1 Health effects categorised due to exposure*

##### 3.2.3.1.1 Acute exposure

These health effects result from exposure to contaminants like pathogens or nitrates that cause an immediate effect. Pathogens are commonly associated with gastrointestinal illnesses that are seldom lethal, but may be lethal in certain sub-populations such as infants. One condition called methemoglobinemia or “blue baby syndrome”, led to the discovery that bacteria in an infant’s intestinal tract can convert nitrates into nitrites, which limits the ability of the haemoglobin of the infant to carry oxygen.

#### 3.2.3.1.2 Chronic exposure

These health effects result from long-term exposure to a contaminant. Contaminants associated with long-term health effects generally are chemicals such as by-products of solvents used by commercial and industrial facilities, pesticides, disinfectants, lead and other metals. Assessment of some disinfection by-products revealed toxicity and even carcinogenicity, while lead exposure is associated with the impairment of mental development in children.

#### 3.2.3.1.3 Critical exposure

Critical exposure health effects result from single or multiple exposures to a contaminant that relate to risk. These critical times of exposure may typically be during early stages of developmental of an infant or an immuno-compromised individual, such as an AIDS patient.

### 3.2.4 Setting MCLGs

After assessing the risk that a contaminant will cause an adverse health effect, the magnitude of this effect must be considered. To assess the magnitude of a health effect, the EPA differentiates only between carcinogenic and non-carcinogenic health effects. A few considerations are taken into account when setting an MCLG. It is important to know whether the effects of a contaminant are first of all carcinogenic. Secondly, the level and mode of exposure that will cause an adverse health effect need to be known. Thirdly, knowledge about sub-population groups that might be more susceptible is essential. Armed with this data, toxicology and epidemiology studies can be performed to confirm the magnitude of the health effects.

#### *3.2.4.1 Toxicology studies*

Toxicology studies involve the study of poisons and their effects. These studies include the introduction of a contaminant to a small sample of test organisms to determine the level of a contaminant that causes adverse health effects. Using mathematical models, scientists can extrapolate the concentration of a contaminant that would cause a single occurrence of a disease in a population of known size. Such a model usually includes safety measures which exaggerate the ability of the chemical. Model organisms are generally used during toxicology studies due to ethical considerations which prevent conducting of harmful experiments on humans.

Toxicology studies are extremely useful because environmental factors (e.g. exposure) can be tightly controlled, which allows for better facilitation and interpretation of results. However, extrapolation of results from animals to humans and from high dose concentrations to environmentally relevant concentrations remains uncertain.

#### *3.2.4.2 Epidemiology studies*

Epidemiology studies are based on the origin of diseases in humans and the circumstances under which the disease originate. Information on disease in humans is gathered from medical records, death certificates

and surveys. The aim of epidemiology is to identify risks and protective factors, with the focus on the causes of disease as a result of environmental exposure. Epidemiology identifies vague relationships between risk factors and disease, where more studies or cases revealing the same relationship make the link more significant or more likely.

Epidemiology studies are extremely valuable when high rates of rare diseases occur in small populations. However, studies become more redundant when focusing on common diseases in large populations. Due to the fact that epidemiology uses data of actual occurrences of disease, there is no need to determine contaminant dose levels. Studies positively confirm the relationship between disease and risk.

The use of these kinds of studies allows the EPA to set MCLGs, but MCLGs only take health goals into account. The value of these goals also needs to be assessed to determine MCLs that are used to regulate the quality of drinking water.

### 3.2.5 Setting MCLs and TTs

As mentioned above, MCLs and TTs are enforceable by laws that govern the quality of drinking water. MCLs are set as close as possible to MCLGs as long as limits are practically feasible. The SDWA defines feasibility as:

- Limits achievable using the best available technique (BAT), TTs or other methods that the EPA propose
- Limits set after inspecting effectiveness in field conditions and not just in a laboratory
- Limits set with giving some thought to affordability

#### *3.2.5.1 Benefit and cost analysis*

According to the SDWA the EPA should set standards for drinking water with the aim to maximize health risk reduction benefits at a cost that is justified by the benefits. Consequently, even though technologies are able to detect extremely low levels of contamination of drinking water, the EPA may decide not to lower the contaminant levels if the cost of lowering the levels is not justified by the benefit gained.

## 3.3 European Union (EU)

The regulation of water quality is one of the most important components of the EU's environmental legislation. The original body of legislation on water was adopted in 1975 with the Surface Water Directive and in 1980 with the Drinking Water Directive (DWD). These directives were based on scientific techniques that were approximately 35 years old, and needed to be updated to the current technologies. In 1998 a new and revised DWD was adopted. Approximately 5 years after this revised directive was enacted, the Commission started making preparations for the next revision, since 25 countries will be involved with the next amendment.

### 3.3.1 Drinking water directive (DWD)

The original DWD legislation consisted of isolated blocks of legislation with no coherent cross-referencing (Hecq et al., 2006; Ochoa-Acuna et al., 2009). In contrast to the original legislation, the current legislation refers to a number of different directives. For example, when the current DWD sets parameters for the concentration of pesticides allowed in water, both the plant protection directive and biocides directive are referenced. The production directive is referenced for the production and distribution of drinking water. Prospective DWDs are intended to be more integrative, which would imply compliance with the requirements of various related directives.

The main focus of the previous DWD was to set standards that protected human health. The aim of the current DWD is to *ensure* human health, and to protect humans from adverse health effects due to the contamination of water. This should be achieved by establishing that the drinking water quality is "wholesome and clean". With drinking water the DWD implies all water consumed by humans, as well as water used in the marketing and production of food. According to the DWD, member states are required to monitor the quality of drinking water and ensure that their water meets the minimum requirements. Furthermore, member states must report to the Commission, as well as making drinking water regulation results available to the public.

### 3.3.2 Parameter set to ensure quality drinking water

Earlier DWDs consisted of 62 "parameters" that contained fields like maximum allowed concentrations (MAC) and Guideline Values (GVs). This large number of parameters complicated earlier versions of the DWD, because not all parameters contained all the possible fields. Also, some of the field values were not scientifically confirmed. Examples of these parameters and their field values are shown in **Error! Reference source not found.**.

After revision of the older DWD, the parameters were limited to a total number of 48 microbial and chemical parameters. These parameters have mandatory fields or parametric values. The WHO guidelines were used when deciding on the parameter values. Both acute effects as well as long term chronic effects were considered when parametric values for various parameters were set. Parameters also compensate for vulnerable groups such as children and pregnant women.

#### *3.3.2.1 Types of water covered by the DWD*

The current DWD covers all water intended for human consumption, except for natural mineral water, medicinal water, and water used in the food industry that does not affect the final product. The directive also enables member states to exempt other water types.

**Table 1. Example of a small number of organic parameters**

| | Method | Principle | Working range[a] | Parametric value[a] | LOD[a] |
|---|---|---|---|---|---|
| **Acrylamide** | No standard method available in CEN/ISO | To be controlled by product specification | | 0.1 | |
| **Benzene** | ISO 11423 (1997a,b) | GC-FID | ≥1 | 1 | 0.25 |
| **Benzo(a)pyrene** | EN/ISO 17993 (2003b) | HPLC-FD | ≥0.005 | 0.01 | 0.0025 |
| **1,2-Dichloroethane** | EN/ISO 10301 (1997d) | GC-ECD | ≥5 | 3 | 0.3 |
| **Epichlorohydrin** | EN 14207 (2003a) | GC-MS | ≥0.1 | 0.1 | |
| | | To be controlled by product specification | | | |
| **Pesticides** | ISO/EN 11369 (1997e) | HPLC-UV | ≥0.1 | 0.1 | 0.025c |
| | EN/ISO 15913 (2003c) | GC-MS | ≥0.05 | | |
| | EN/ISO 6468 (1996) | GC-ECD | ≥0.01 | | |

[a] ECD is electron capture detection; FD is fluorescence detection; FID is flame ionisation detection; GC is gas chromatography; HPLC is high performance liquid chromatography; LOD is the limit of detection, to be achieved according to Council Directive 98/83/EC, 1998; MS is mass spectrometry; Parametric value is the required limit according to Council Directive 98/83/EC, 1998; UV is ultra violet detection; Working range is the lowest determinable concentration stated in the method.

[b] Several organic insecticides, herbicides, fungicides, nematocides, acaricides, algicides, rodenticides, slimicides their relevant metabolites, degradation and reaction products. Only those pesticides which are likely to be present in a given supply need be monitored (Council Directive 98/83/EC, 1998).

[c] The LOD applies to each individual pesticide and may not be achievable for all pesticides at present (Council Directive 98/83/EC, 1998).

#### *3.3.2.2 Microbial parameters*

Since the presence of microorganism in drinking water generally implies an adverse health effect, the parameter values are set to zero.

#### *3.3.2.3 Carcinogenic parameters*

Carcinogens typically have no thresholds at which they pose no risk to human health. The WHO criterion for acceptable disease burden of carcinogens is based on a DALY value of $10^{-6}$, i.e., there should be no more than one excess cancer case in a population of $10^5$ resulting from a lifetime of exposure to the water. The DWD uses an even stricter DALY value of $10^{-7}$.

#### *3.3.2.4 Other considerations*

A practical consideration when setting parametric values is the availability of analyses and detection methods capable of quantifying low levels of contaminant. The process of setting parametric values should also take into account whether the available treatment techniques are sufficient to achieve MACs. A balance needs to be reached between risk to human health when consuming water that does not meet the minimum requirements set by the DWD, and risk due to an interruption in the water supply.

### 3.3.3 Sampling and monitoring contaminants

The DWD suggest two kinds of monitoring methods: occasional and periodic. Occasional monitoring is performed where only a small population size is supplied with drinking water. Periodic monitoring is performed with water systems that supply bigger communities. The current DWD focuses on monitoring of tap water. Risk assessment and management based approaches may well improve the way parameters are monitored and sampled in future.

### 3.3.4 Quality control and assurance

The previous DWD quality control was limited to referenced monitoring methods only. The current DWD makes use of ISO/CEN methods, and the definition of performance criteria for chemico-physical parameters. Member states are required to have QA/QC systems in place for drinking water analysis. The purposes of these QA/QC methods are not exclusively to monitor parameters, but also the gathering of information on the performance of the water treatment.

## 3.4 United States Geological Survey (USGS)

The USGS is a US government organisation that studies the natural environment, resources and natural hazards that may pose a threat to the US population. The USGS concentrates on four major scientific disciplines. These are biology, geography, geology and hydrology. The USGS' primary focus is to gather knowledge through research rather than assuming the role of regulating potential hazards.

The USGS started surveying water in 1991, when they established National Water Quality Assessment (NAWQA). Under the NAWQA the USGS systematically gathered chemical, biological and physical water quality data from 42 sources of water across the US. Currently the USGS NAWQA contains data about:

- Concentration of chemicals in water supplies and surface water, as well as information about the presence of chemicals in aquatic organisms
- Geological information as well as complex features with many descriptive variables
- Regular inflow of information from predetermined sampling sites
- Water table level for sampled wells
- Information about 7300 surface water sites and 9800 wells
- Information about nutrient samples, pesticide samples and Volatile Organic Compound samples
- Samples of aquatic organism tissues and bed sediment
- Data about aquatic organisms and environments, including aquatic macro-invertebrate and algae

The USGS also initiated a project called the "Emerging Contaminants Project" to compile information on the potential threat of ECs to human health. This project is composed of five main thrusts:

- Development of methods to quantitate chemicals and microorganisms in drinking water and in the environment

- The determination of the occurrence of ECs in the environment
- A description of the paths by which ECs find their way into drinking water and the environment
- A description of the processes that contribute to EC transport in the environment
- Identification of ecological effects due to exposure to ECs

These surveys compile information about water quality conditions over time, and how human intervention affects these conditions. Data monitored in this way are integrated with geological information to aid the understanding of water quality of unmonitored areas.

The US uses NAWQA to aid the design and implementation of strategies that focus on managing, protecting, and monitoring quality of water resources. A list of contaminants proposed for regulation can be found in the USGS list of probable contaminants in drinking water (Appendix C).

## 3.5 National Institute of Environmental Health Sciences (NIEHS)

The NIEHS is a division of the National Institutes of Health (NIH), which is a division of the Department of Health and Human Services (DHHS) of the United States. The NIEHS was established with the aim to gain more knowledge on environmental influences that might have adverse effects on humans. The NIEHS therefore concentrates on disease-orientated research, clinical research, environmental health studies, and the training of multi-disciplinary researchers. The institute also supports centres for environmental health studies at universities across the United States. The NIEHS is not directly involved in the establishment of allowed contaminant levels or the development of regulations that manage drinking water quality.

## 3.6 Conclusions

The EPA periodically releases a Contaminant Candidate List (CCL). The decision on whether to include a compound in the CCL is taken based on three key considerations:

1. Does the contaminant pose a health risk to consumers?
2. Is the contaminant likely to occur at a concentration of concern?
3. Is there a meaningful opportunity for health risk reduction?

A positive response to all three questions typically qualifies a compound for inclusion in the CCL, which then mandates regulation of the compound. An important aspect of this program is the continual assessment of the exposure of consumers to ECs in drinking water. This depends on the periodic monitoring of the occurrence of a given compound in water, a task that is often performed by independent agencies, such as the United States Geological Survey. A careful assessment of toxicological and epidemiological data then allows the establishment of guideline values, a concentration level where the health impact of an EC in a population is regarded as negligible.

A similar approach is followed in the EU with the DWD, which is also based on monitoring programs and the establishment of guideline values. Once guideline values have been established, periodic monitoring is used to assess whether the quality of the water conforms to the established guidelines.

# 4 Identification of ECs in South African Drinking Water

## 4.1 A limited scoping study for ECs in drinking water in two South African cities

In order to gain an insight into the range of possible ECs present in drinking water in South Africa, we undertook a limited, qualitative screen of drinking water in two cities. This was performed between January 2010 and July 2011, a period that partially overlapped with the current WRC project. We sampled drinking water directly from taps in Johannesburg and in Bloemfontein. Samples were taken at different times a few months apart to include possible seasonal variation. We utilised LC-MS-MS capable of detecting more than 600 different polar, water-soluble compounds (listed in Appendix D), including pharmaceuticals, pesticides, endocrine disruptors and cyanotoxins. The aim of the exercise was to develop a rough list of possible ECs to eventually quantitate as part of the national survey. This was to avoid the possibility of performing an extensive quantitative screen on an EC regarded as critical in Europe or the US, but which did not occur in South African drinking water. The aim was therefore to evaluate the rough list of ECs detected in tap water in at least two major cities for the severity of the health impact of each, before making a final choice of ECs to survey. The results of the qualitative screen are shown in Table 2.

**Table 2. Results of a limited qualitative screen of pharmaceuticals and pesticides in drinking water in two South African cities. A solid circle indicates the presence of a compound.**

| Determinant | Location | Bloemfontein | | | | | | Johannesburg | | Positive samples (%) | Hazard[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Date | Jan 2010 | Oct 2010 | Jan 2011 | May 2011 | Jul 2011(1) | Jul 2011(2) | Dec 2010 | Jun 2011 | | |
| Amphetamine | | • | • | | • | • | | | • | 63 | L |
| Atrazine[a] | | | • | • | | | • | • | • | 63 | H |
| Carbamazepine | | • | • | • | | • | | • | | 63 | H |
| Diphenylamine | | | | | | | | | • | 13 | L |
| Imidacloprid | | | | | | | | | • | 13 | L |
| Metolachlor[a] | | | | • | • | • | • | • | • | 75 | U |
| Oxadixyl | | | • | | | | | | | 13 | L |
| Simazine* | | | | | | | | | • | 13 | L |
| Tebuthiuron | | | | | • | • | • | • | • | 63 | L |
| Telmisartan | | | • | | | | | | | 13 | H |
| Terbuthylazine[a] | | • | • | • | • | • | • | • | • | 100 | L |

[a] Guideline values are specified for these determinants in drinking water ((World Health Organization, 2011d))

[b] H: high; L: low; U: unknown. These assessments are based on the peer-reviewed epidemiology studies in the Hazardous Substances Databank (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB). High and low hazard substances were shown to have severe or limited health impacts, respectively.

Referring to Table 2, a total of 11 pesticides or pharmaceuticals were detected over the sampling period. Of these, 6 compounds were detected in more than half of the samples. These 6 compounds were amphetamine, atrazine, carbamazepine, metolachlor, tebuthiuron, and terbuthylazine. We evaluated peer-reviewed data on the acute toxicity and epidemiology of each of the 11 identified compounds. The data are presented in Table 3.

**Table 3. The use, acute toxicity and epidemiology of compounds detected in drinking water in Bloemfontein and Johannesburg.**

| **Amphetamine** |
|---|
| **Use**<br>Central nervous system stimulant, anorexic. |
| **Toxicity**<br>Doses as little as 2 mg, but more likely between 15 and 30 mg, may induce toxic effects. However, even doses of 400-500 mg are not uniformly fatal (Budavari, 1996; Munger et al., 1997a). The acute lethal dose in adults has been reported at 20-25 mg/kg, and in children, 5 mg/kg. Death from as little as 1.5 mg/kg in an adult has also been noted (Gossel and Bricker, 1994; Paneth, 1995). |
| **Epidemiology**<br>Amphetamines used in large doses over a long period of time may lead to substantial weight loss, liver disease, hypertensive disorders, kidney damage, stroke, heart attack, non-healing ulcers, and sores in the skin (Swan, 2006; Young and Koda-Kimble, 1995). |
| **Atrazine** |
| **Use:**<br>Herbicide to control broadleaf and grassy weeds (Humburg, 1989; USEPA). |
| **Acute Toxicity**<br>Ingestion of 100 g (not mg) of atrazine may lead to coma, circulatory collapse, metabolic acidosis, and gastric bleeding, which can be followed by renal failure, hepatic necrosis, and a disseminated intravascular coagulopathy that may be fatal (Ellenhorn et al., 1997b; McEvoy, 2007). |
| **Epidemiology**<br>Elevated levels of atrazine were found in drinking water in the rural community of Rathbun in Iowa. A comparison of the rates of low birth weight, prematurity, and intrauterine growth retardation (IUGR) in live singleton births during the period 1984-1990 by women living in 13 communities served by the Rathbun water system were compared to other communities of similar size in the same Iowa counties. It was found that the Rathbun communities had a greater risk of intrauterine growth retardation than southern Iowa communities with other surface sources of drinking water (relative risk = 1.8; 95% CI = 1.3, 2.7). In addition, multiple linear regression analyses revealed that levels of the herbicide atrazine, metalochlor, and cyanzinc were each significant predictors of community intrauterine growth retardation rates (Munger et al., 1997b; Takamiya et al., 2006). Furthermore, two studies from northern Italy showed elevated risks of ovarian tumours among women exposed to triazine herbicides including atrazine, suggesting a need to review the carcinogenic classification of this compound (Bingham et al., 2001a; Chevrier et al., 2011). In a study in Ontaria, atrazine contamination levels (range 50-649 ng/L) were positively associated with stomach cancer incidence and negatively associated with colon cancer incidence (Matalon et al., 2002; Van Leeuwen et al., 1999a; Wulfeck-Kleier et al., 2010). |

| Carbamazepine |
| --- |
| **Use**<br>Therapy. Analgesic, Anticonvulsant |
| **Toxicity**<br>A case study of 14 children who ingested carbamazepine showed that with serum levels from 18 ug/mL to 32 ug/mL, nystagmus and drowsiness were the most common signs of overdose. |
| **Epidemiology**<br>A significant increase in the occurrence of malformations was found in a study of 1256 children following pre-natal exposure to anti-epileptic drugs (valproic acid or carbamazepine) (Scolnik et al., 1994; Wide et al., 2004). |
| **Diphenylamine** |
| **Use**<br>Herbicide, fungicide, manufacturing of dyes, industrial anti-oxidant |
| **Toxicity**<br>May be irritating to mucous membranes. |
| **Epidemiology**<br>None |
| **Imidacloprid** |
| **Use**<br>Insecticide |
| **Toxicity**<br>A 4-year-old child who ingested imidacloprid at 10 mg/kg bodyweight showed no signs of poisoning or adverse health effects. |
| **Epidemiology**<br>None |
| **Metolachlor** |
| **Use**<br>Pesticide. Control of annual grasses and some broad-leaved weeds |
| **Toxicity**<br>Possible human carcinogen |
| **Epidemiology**<br>None |
| **Oxadizyl** |
| **Use**<br>Fungicide |
| **Toxicity**<br>LD50 560 mg/kg bodyweight (mouse) |

| |
|---|
| **Epidemiology**<br>None |
| **Simazine** |
| **Use**<br>Herbicide |
| **Toxicity**<br>LD50 970 mg/kg bodyweight (rat) |
| **Epidemiology**<br>No correspondence to cancer |
| **Tebuthiuron** |
| **Use**<br>Broad-spectrum herbicide for control of herbaceous and woody plants. |
| **Toxicity**<br>LD50 579 mg/kg bodyweight (mouse) |
| **Epidemiology**<br>None |
| **Telmisartan** |
| **Use**<br>Anti-hypertensive (angiotensin II receptor antagonist) |
| **Toxicity**<br>No information |
| **Epidemiology**<br>At least 15 case reports describe oligohydramnios, fetal growth retardation, pulmonary hypoplasia, limb contractures, and calvarial hypoplasia in various combinations in association with maternal telmisartan treatment during the second or third trimester of pregnancy. Stillbirth or neonatal death is frequent in these reports, and surviving infants may exhibit renal damage. |
| **Terbuthylazine** |
| **Use**<br>Herbicide to control a wide range of weeds |
| **Toxicity**<br>LD50 1 845 mg/kg bodyweight (rat) |
| **Epidemiology**<br>Terbuthylazine is mildly to moderately irritating to the eyes, and slightly irritating to the skin. |
| [a] *Data obtained from the Hazardous Substances Database (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB) and ChemIDPlus Lite (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CHEM).* |

## 4.2 Choosing ECs for a national, quantitative survey

Typically, in drinking water, acute toxicity is not a key concern, except in the case of a major chemical spill into the source, in which case municipal emergency response measures are usually triggered. The extended exposure to low levels of contamination and its possible impact on human health is a greater concern in the management of water quality. The epidemiology of each contaminant, particularly were it involves drinking water, is thus extremely relevant when considering the possible health risks of each contaminant. Referring to Table 3, it is seen that 6 of the compounds identified in the limited qualitative screen had associated epidemiology studies. These studies suggested that atrazine, carbamazepine and telmisartan posed the greatest health risk. Atrazine was found to be associated with an increase in intra-uterine growth retardation (IUGR) as well as ovarian and stomach cancer in contaminated water samples. The anticonvulsant drug, carbamazepine, was shown to be associated with an increased chance of developmental malformation when foetuses were exposed to the drug during maternal therapy. It is not clear what the effective exposure levels were in these studies. Atrazine and carbamazepine were detected in 63% of the drinking water samples taken in Bloemfontein and Johannesburg. The exposure of foetuses to the anti-hypertension drug, telmisartan, during the second and third trimester of pregnancy, was also reported to be associated with IUGR as well as with stillbirths, neonatal deaths and renal damage in surviving infants. However, telmisartan was detected only once in water from Bloemfontein. Terbuthylazine, on the other hand, was detected in 100% of the samples. Although the epidemiological data on terbuthylazine suggested that it posed a lesser health risk, the frequency of detection of this herbicide warranted closer attention.

There were no published epidemiology studies available for 5 of the compounds identified in the drinking water. Thus, in terms of the known peer-reviewed health effects and detection frequency, atrazine, carbamazepine and terbuthylazine are of concern. Thus, in terms of the occurrence in drinking water and the severity of the possible health effects, we proposed that the herbicide atrazine, the anticonvulsant drug, carbamazepine, and the herbicide terbuthylazine should be quantitated in South-African drinking water over a one year period (four seasons). Such a quantitative study will allow an informed assessment of the potential health risks posed by these three compounds.

# 5 Overview of Methods for the Detection and Quantitation of ECs

Over the last decade pharmaceuticals and pesticides have become a public concern due to a greater awareness of the presence of such contaminants in drinking water. Because of concerns to public health, sensitive and reliable tools and methods to monitor ECs are continuously being developed and refined. Liquid chromatography coupled with mass spectrometry has emerged as the predominant technique to screen and quantitate polar ECs in drinking water. A discussion of this technique is given below. Emphasis is placed on methods used for the analysis of the ECs atrazine (ATR), terbuthylazine (TBA) and carbamazepine (CBZ), which will be quantitated in the national reconnaissance study.

## 5.1 Pesticides

A review of the literature showed that HPLC-MS was the predominant method used to analyse ATR, TBA and CBZ contaminants in an aquatic matrix (Carvalho et al., 2008). In the study by *Wille et al*. a method to rapidly quantify pesticides and pharmaceuticals was described (2011). Chromatography was carried out using Ultra-HPLC and a high speed LC fitted with a Nucleodur C18 Pyramid column (1.8 µm, 100 mm × 2 mm). Mass spectrometric analysis was performed using an orbitrap-based mass spectrometer, the Exactive Benchtop MS (Thermo Scientific). The parameters used for optimum ionisation of pesticides and pharmaceuticals are shown in Table 4.

**Table 4. Parameters of MS for optimal ionisation**

| | Pharmaceuticals | Pesticides |
|---|---|---|
| Spray voltage (kV) | 4.0 | 4.0 |
| Sheath gas flow rate (arbitrary units, au) | 30.0 | 30.0 |
| Auxiliary gas flow rate (au) | 0.0 | 0.0 |
| Capillary temperature (◦ C) | 275.0 | 250.0 |
| Heater temperature (◦ C) | 250.0 | 350.0 |
| Capillary voltage | 82.5 (−30.0) | 82.5 (−30.0) |
| Tube lens voltage | 170.0 (−95.0) | 120.0 (−95.0) |

*Wille et al*. (2011) validated their method according to guidelines of the Commission Decision 2002/657/EC for quantitative confirmation (EU), and the guidelines of SANCO/10684/2009 on pesticide residues analysis (CD; SANCO, 2009).

## 5.2 Pharmaceuticals

In a study by Kim et al. (2011), an approach was developed to use reverse-phase (RP) HPLC chromatographic separation coupled with MS-based Multiple Reaction Monitoring (MRM). MRM is a tandem mass spectrometric method that is used during quantitation of analytes, and involves the rapid toggling between the detection of specific precursor and fragment pairs that are unique to a compound. The principle behind MRM is that both the precursor as well as the most abundant fragment of an analyte must be detected at

the correct elution time for an analyte to be regarded as being present in a matrix (Cox et al., 2005). The quantitative level of the compound of interest is determined from the area of the elution peak recorded during the period when the characteristic MRM transitions were observed (Hernando et al., 2007).

During their analysis *Kim et al.* aimed to establish a fully validated, fast and precise LC-MS/MS method to quantitate frequently-prescribed pharmaceuticals that may occur in drinking water. Stock solutions were prepared for each standard and were used to set up calibration curves. Linear regression with a weighting factor was applied to the calibration curves. The deuterated internal standards $d_{10}$-phenytoin and $d_6$-valproic acid were also used to correct for possible matrix effects. Quality control samples (QCs) were used to confirm method accuracy, precision and sample stability. QC samples were prepared at four different concentrations: at the lower limit of quantitation (LLOQ), low, medium and upper limit of quantitation (ULOQ) levels. A pre-analysis was performed to determine the degree of ionisation of each analyte under positive as well as negative ionisation conditions, and the most abundant fragment of each analyte was determined. After sample preparation, the samples were analysed using ESI-LC-MS. Chromatography was performed using an Agilent 1100 series HPLC fitted with a Luna C18 column (100 × 2.0 mm, 3 µm). MS analysis was performed with a triple quadrupole QTRAP 4000 (SciEx) mass spectrometer. MS parameters were 40, 50, and 20 psi for the nebulising, turbo spray and curtain gas, respectively, while the turbo gas temperature was set to 600°C. The ESI needle voltages were set to 5500 V and -4500 V for positive- and negative-ion modes, respectively. Data were analysed using Analyst 1.4.1 software (SciEx).

Method validation was carried out according to the FDA guidelines (FDA). To test selectivity, 6 sample blanks were analysed to ensure that no peaks were present at the relevant retention times. Accuracy, precision and stability were determined using the QC samples. Recoveries were calculated as a function of percentage of the ratio between the mean peak area of an analyte spiked before extraction compared to the mean peak area of the same analyte spiked directly into methanol at the same concentration.

We followed a similar approach in setting up our method validation.

## 5.3 FDA guidelines for method development and validation

The development and validation of a rapid method to quantitate the levels of three ECs, atrazine (ATR), terbuthylazine (TBA) and carbamazepine (CBZ), were performed as prescribed by the Food and Drug Administration (FDA, 2001).

The Guidelines of the FDA refers to three types of validation: full, partial, and cross validation. Full validation is crucial during the development of a novel method that aims to detect or quantitate a newly discovered compound. Partial validation is the process where an existing method is modified to suit a more specific investigation of analytes or to apply a method to different instruments or techniques available in a laboratory. During partial validation it is the analyst's responsibility to decide where validation for the modified method is required. Cross-validation includes the comparison between the results of different techniques.

The validation and development of an analytical method requires correct and rigorous documentation. This documentation can be divided into the following sections: reference standard preparation, bioanalytical method development and the application of the validated bioanalytical method to define the acceptance criteria for an analytical run.

Each of these sections is addressed separately below.

### 5.3.1 Reference standards preparation

Reference standards are instrumental during the development and validation of bioanalytical methods. These standards are used to set up reference calibration curves and quality control samples. Consequently, information about these standards must be well established, and knowledge of parameters such as standard identity, purity, source, lot number and expiry dates are essential to ensure robustness of a method.

### 5.3.2 Key parameters for method validation

A valid method must meet certain criteria; these criteria are described using key parameters such as accuracy, precision, selectivity, sensitivity, reproducibility and stability. These parameters are discussed individually below.

- **Selectivity** is defined as the ability of a method to discriminate between different analytes within the same matrix, as well as the ability to effectively measure an analyte's concentration within a biological matrix.
- **Accuracy** is defined as the proximity of the mean result of repetitive determinations to the true concentration of a known analyte. Acceptable accuracy is defined as a minimum mean value deviation of 15%, with the exception of allowing a 20% deviation at the LLOQ. Accuracy is expressed as the *bias* (equation **2**) from the true concentration.

$$bias\left[\widehat{\theta}\right] = E\left[\widehat{\theta}\right] - \theta, \text{ averaging over all possible observations} \qquad \text{(Eq. 2)}$$

Where $E[\widehat{\Theta}]$ denotes the expected value and Θ the actual measurement.

- **Precision** is defined as a measure of the ability the method to replicate results using aliquots from the same homogeneous biological matrix. Precision is calculated using the coefficient of variation (Cv) as shown in equation 3**.**

$$C_v = \frac{\sigma}{\mu} \qquad \text{(Eq. 3)}$$

The maximum $C_v$ at each concentration level should not be greater than 15% with the exception of the LLOQ, where the $C_v$ should not exceed 20%.

- **Recovery** is a measure that indicates the efficiency of the extraction of analytes from a biological matrix. Recovery is determined by comparing the detected quantity of a known spiked amount of analyte extracted from a biological matrix with the response from an equal spiked quantity detected without extraction. Recovery is often accompanied with the introduction of an internal standard (IS). An IS is introduced to correct potential effects that a matrix may have on analytes. ISs should not occur naturally in the biological matrix, but must be biochemically similar to the analytes to be quantitated. An IS is generally chosen so that the ratio of the recovery of the IS and the analyte of interest is constant; this relationship between the IS and analytes is essential for accurate quantitation. A recovery threshold of > 40% is normally acceptable.
- **Stability** is a function of the effects that environmental changes have on chemical standards and biological matrices during sample collection, analysis and sample storage. Three different stability tests are typically performed in method validation:
    - *Short-term temperature stability test*: This test determines the stability of an analyte in a biological matrix. In nature analytes may be present at low or high concentrations. Representative aliquots that reflect these possibilities are therefore used to test stability. Two samples are prepared using Milli-Q water spiked with a mixture of high and low concentrations of standards, respectively.
    - Each of the samples is analysed and then left for 6 h at 22 °C. After this period the stability of the samples are determined by re-analysing the samples and comparing the analyte peak areas. After the second analysis the samples are left for an additional 18 h (24 h in total). After this period the stability is re-analysed, and peak areas of the three analyses compared. If little to no deviation in peak area occurred, analytes are considered to be stable.
    - *Stock Stability test*: Analyte standards are typically prepared in stock solutions. The standard may not be stable in the stock solution, resulting in incorrect calibration curves after prolonged storage of the stock solutions. To address this possibility, stock solutions are also analysed directly, after 6 h and after 24 h. Stock solutions are prepared by spiking a mixture of analytes into methanol. After 6 or 24 hours the stability of the analyte is analysed in the stock solutions by comparing the peak area of the initial analysis, with the peak area of analysis after 6 and 24 h of storage, respectively.
    - *Sample stability during analysis*: This test determines the stability of samples while they are inside the auto-sampler of the HPLC instrument. Similar to other stability tests, two samples are analysed immediately, after 6 h, and after 24 h in the auto-sampler. The peak areas of each of the analytes are compared to determine any instability during the period that the samples spent in the auto-sampler.

These fundamental principles of method validation play a vital role in the development of a valid and accurate assessment method. Validation assures reliable results throughout the analytical process of screening and quantification of ECs in a biological matrix. The methodologies applied during methods validation of our quantitation protocol will be discussed more thoroughly below.

# 6 Method Development and Validation

In the previous section we discussed the different parameters used to validate a quantitation method. In this section we will discuss the steps involved in assessing these parameters.

## 6.1 Selectivity and interference

According to the FDA selectivity can be verified by confirming that a blank sample does not exhibit analyte peaks at pre-determined LC retention times. We extended this step by also determining if ECs interfered with each other during ionisation and quantification. Analysis of selectivity and interference were therefore subdivided into two separate analyses.

During the first part of the analysis 3 vials were filled with $ddH_2O$/0.1% Formic Acid (FA) containing 50 µg/L of ATR, TBA or CBZ. A fourth vial was filled with a 50 µg/L mixture of ATR, TBA and CBZ in $ddH_2O$/0.1% FA, and a fifth vial contained only $ddH_2O$/0.1% FA and acted as blank. The determination was repeated 3 times to assure precision and reliability of the analysis.

Each of the vials were analysed using HPLC-MS (Agilent 1200 series coupled with an AB Sciex 3200 QTRAP). To determine possible interference, the replicate average peak areas of each analyte in the mixture sample were compared with the replicate average peak areas of the samples containing a single analyte only (see Table 5).

**Table 5. Interference and the coefficient of variance (Cv) for each MRM of ATR, CBZ and TBA (the MRM fragment sizes are also given in Table 10).**

ATR

| Repeat 1 | ATR 1 | Mix 1 | Cv |
|---|---|---|---|
| Atrazine (*m/z* 174.100) | 525000 | 524667 | 0.04% |
| Atrazine (*m/z* 104.000) | 183000 | 183000 | 0.00% |
| Repeat 2 | ATR 2 | Mix 2 | Cv |
| Atrazine (*m/z* 174.100) | 573667 | 578667 | 0.61% |
| Atrazine (*m/z* 104.000) | 202667 | 203667 | 0.35% |
| Repeat 3 | ATR 3 | Mix 3 | Cv |
| Atrazine (*m/z* 174.100) | 541000 | 532667 | 1.10% |
| Atrazine (*m/z* 104.000) | 190000 | 185667 | 1.63% |

CBZ

| Repeat 1 | CBZ 1 | Mix 1 | Cv |
|---|---|---|---|
| Carbamazepine (*m/z* 194.200) | 167667 | 160333 | 3.16% |
| Carbamazepine (*m/z* 192.100) | 39067 | 37067 | 3.72% |
| Repeat 2 | CBZ 2 | Mix 2 | Cv |
| Carbamazepine (*m/z* 194.200) | 120667 | 128333 | 4.35% |
| Carbamazepine (*m/z* 192.100) | 27933 | 29233 | 3.22% |
| Repeat 3 | CBZ 3 | Mix 3 | Cv |
| Carbamazepine (*m/z* 194.200) | 128000 | 125000 | 1.68% |
| Carbamazepine (*m/z* 192.100) | 29367 | 29100 | 0.65% |

TBA

| Repeat 1 | TBA 1 | Mix 1 | Cv |
|---|---|---|---|
| Terbuthylazine (*m/z* 174.100) | 1083333 | 1023333 | 4.03% |
| Terbuthylazine (*m/z* 104.000) | 192000 | 184667 | 2.75% |
| Repeat 2 | TBA 2 | Mix 2 | Cv |
| Terbuthylazine (*m/z* 174.100) | 1056667 | 1146667 | 5.78% |
| Terbuthylazine (*m/z* 104.000) | 187667 | 206667 | 6.81% |
| Repeat 3 | TBA 3 | Mix 3 | Cv |
| Terbuthylazine (*m/z* 174.100) | 1031333 | 972667 | 4.14% |
| Terbuthylazine (*m/z* 104.000) | 182000 | 176333 | 2.24% |

CBZ, ATR and TBA could not be detected at the retention times of 6.3, 6.5 and 6.7 min, respectively, in the sample blanks, demonstrating method selectivity. The results also clearly showed that ATR, CBZ and TBA did not interfere in the quantitation when present in the same sample mixture, suggesting that these compounds could successfully be quantitated in natural water samples.

We then proceeded to test for the possible interference between ATR, CBZ and TBA during analyte extraction. Since we will be making use of active sampling during the national reconnaissance study, involving solid phase extraction, we wanted to establish that the combined presence of ATR, CBZ and TBA in a sample did not interfere with individual extraction efficiencies. To this end, three 1 L bottles were spiked with 50 ng/L of ATR, TBA and CBZ respectively in 1 L dd$H_2O$. A fourth bottle was spiked with a mixture of 50

ng/L ATR, TBA and CBZ in 1 L dd$H_2O$, while a fifth bottle, the blank, contained 1 L of dd$H_2O$. The analytes were extracted using Strata 18-CE cartridges and reconstituted within 1 mL dd$H_2O$/0.1% FA. This action was repeated three times to determine precision and reliability. Interference was determined by comparing the replicate average peak areas of each analyte in the mixture with that in the samples containing only a single analyte (Table 6).

**Table 6. Interference and the coefficient of variance (CV) for each MRM of ATR, CBZ and TBA in extracted samples (the MRM fragment sizes are also given in Table 10).**

Extracted ATR

| Repeat 1 | ATR 1 | Mix 1 | Cv |
|---|---|---|---|
| Atrazine (*m/z* 174.100) | 572250 | 524667 | 6.13% |
| Atrazine (*m/z* 104.000) | 199470 | 183000 | 6.09% |
| | | | |
| Repeat 2 | ATR 2 | Mix 2 | Cv |
| Atrazine (*m/z* 174.100) | 596613 | 624960 | 3.28% |
| Atrazine (*m/z* 104.000) | 202667 | 219960 | 5.79% |
| | | | |
| Repeat 3 | ATR 3 | Mix 3 | Cv |
| Atrazine (*m/z* 174.100) | 543461 | 532667 | 1.42% |
| Atrazine (*m/z* 104.000) | 201537 | 185667 | 5.80% |

Extracted CBZ

| Repeat 1 | CBZ 1 | Mix 1 | Cv |
|---|---|---|---|
| Carbamazepine (*m/z* 194.200) | 63713 | 67340 | 3.91% |
| Carbamazepine (*m/z* 192.100) | 14455 | 14827 | 1.80% |
| | | | |
| Repeat 2 | CBZ 2 | Mix 2 | Cv |
| Carbamazepine (*m/z* 194.200) | 43440 | 48767 | 8.17% |
| Carbamazepine (*m/z* 192.100) | 10056 | 11401 | 8.86% |
| | | | |
| Repeat 3 | CBZ 3 | Mix 3 | Cv |
| Carbamazepine (*m/z* 194.200) | 52880 | 53750 | 1.15% |
| Carbamazepine (*m/z* 192.100) | 12040 | 12555 | 2.96% |

Extracted TBA

| Repeat 1 | TBA 1 | Mix 1 | Cv |
|---|---|---|---|
| Terbuthylazine (*m/z* 174.100) | 1102536 | 1056685 | 3.00% |
| Terbuthylazine (*m/z* 104.000) | 195237 | 188941 | 2.32% |
| | | | |
| Repeat 2 | TBA 2 | Mix 2 | Cv |
| Terbuthylazine (*m/z* 174.100) | 1075667 | 1146667 | 4.52% |
| Terbuthylazine (*m/z* 104.000) | 197667 | 215357 | 6.06% |
| | | | |
| Repeat 3 | TBA 3 | Mix 3 | Cv |
| Terbuthylazine (*m/z* 174.100) | 1098973 | 1035768 | 4.19% |
| Terbuthylazine (*m/z* 104.000) | 182154 | 168648 | 5.44% |

These results clearly showed that each of ATR, CBZ and TBA could reliably be extracted from 1 L water samples irrespective of the presence of the other compounds.

## 6.2 Calibration curves

We have established above that there was negligible interference between ATR, CBZ and TBA during the MRM-based LC-MS quantitation or during sample extraction. We thus proceeded with a sample containing a mixture of these three compounds in the subsequent establishment of calibration curves.

A calibration curve was set up using dilutions of a mixture of the ECs. Dilutions of 1, 0.5, 0.25, 0.1, 0.05, 0.01, 0.005, 0.001, 0.0005, 0.0001, 0.00005 and 0.000025 mg/L were used to plot an initial calibration curve. From this curve the Lower Limit Of Quantitation (LLOQ) of ATR, TBA and CBZ were determined at 100 ng/L, 50 ng/L and 50 ng/L respectively (see Figure 3-Figure 5). The LLOQ was defined as the concentration with a minimum signal-to-noise (S/N) ratio of at least 10. A line was fitted by linear regression to each data group of values for each compound, and the upper limit of quantification (ULOQ) was determined to be at 100 µg/L (see Figure 6-Figure 8). It was thus decided that working calibration curves will be determined in the range 50 ng/L to 100 µg/L.

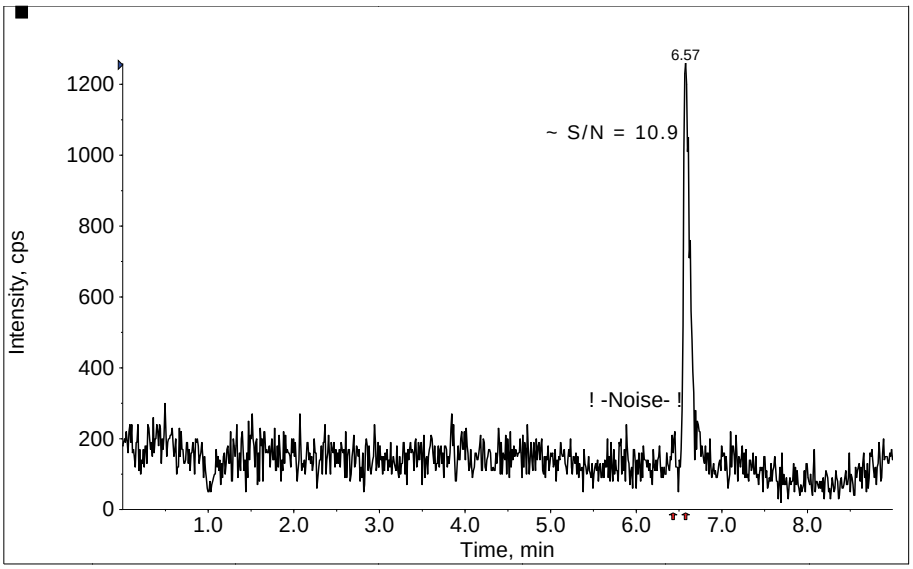


**Figure 3. Determination of LLOQ for ATR. The MRM transition 216/174, specific for ATR, was followed during LC of the sample. A signal-to-noise (S/N) ratio of approximately 11 for the major MRM peak of ATR and a representative part of the background (indicated by the arrows) was observed at an ATR concentration of 100 ng/L.**

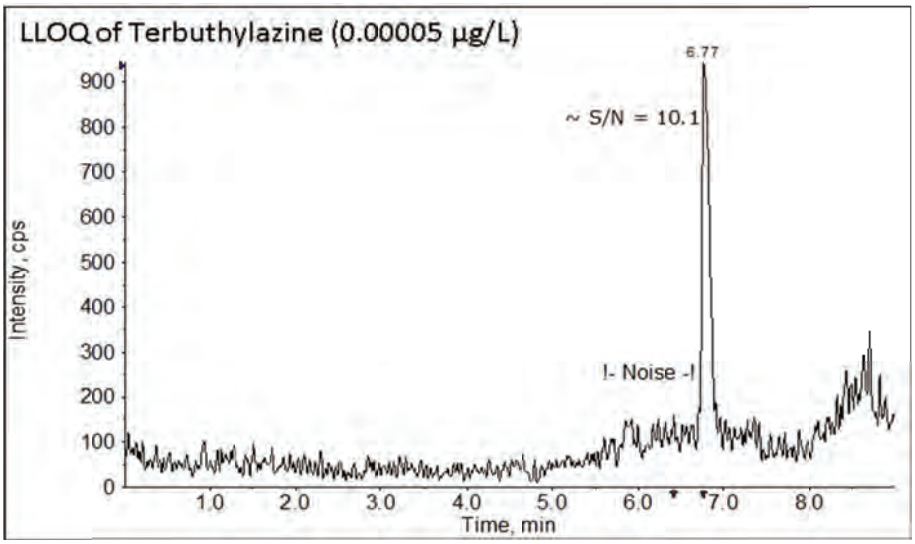


**Figure 4. Determination of LLOQ for TBA. The MRM transition 230/174, specific for TBA, was followed during LC of the sample. A signal-to-noise ratio (S/N) of approximately 10 for the major MRM peak of TBA and a representative part of the background (indicated by the arrows) was observed at a TBA concentration of 50 ng/L.**

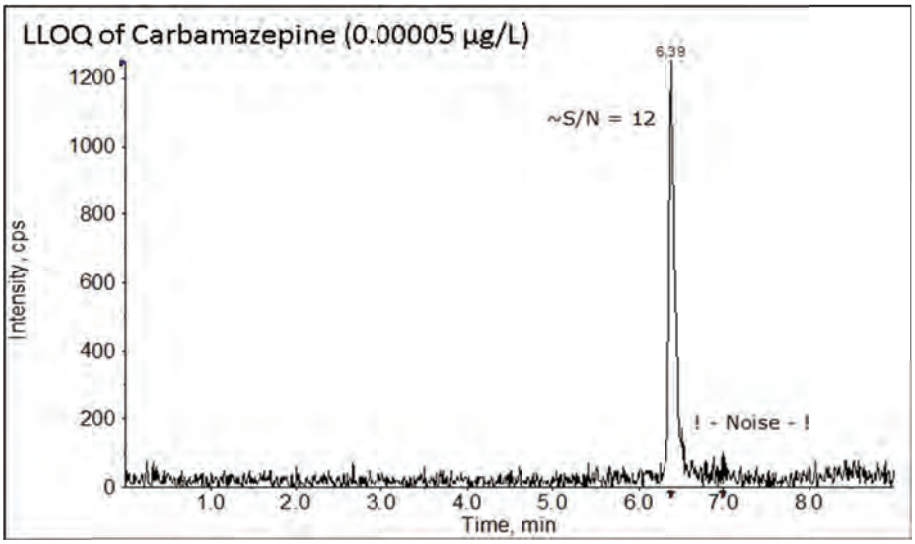


**Figure 5. Determination of LLOQ for CBZ. The MRM transition 237/194, specific for CBZ, was followed during LC of the sample (A). The total ion count shown in (A) was smoothed (B). A S/N of approximately 12 for the major MRM peak of CBZ and a representative part of the background (indicated by the arrows) was observed at a CBZ concentration of 50 ng/L.**

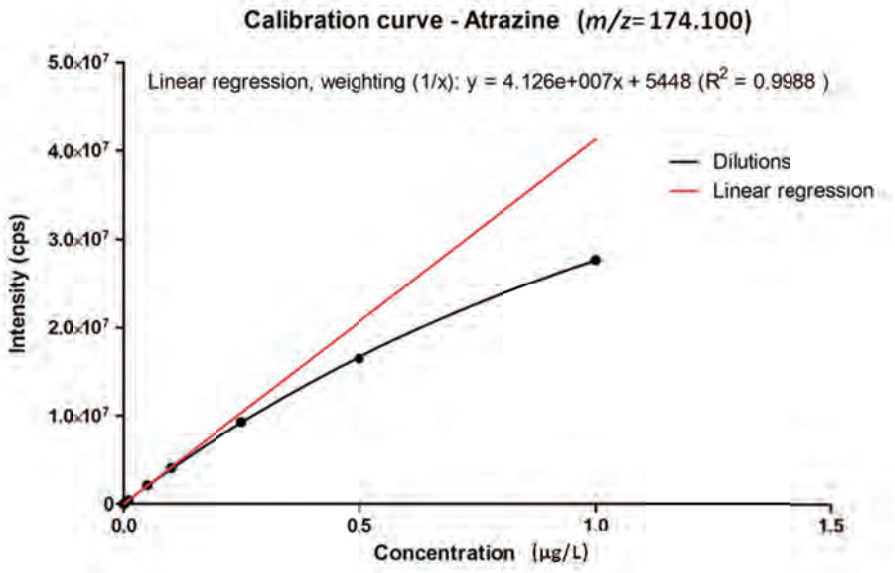


**Figure 6. Determination of ATR ULOQ using linear regression and 1/x weighting**

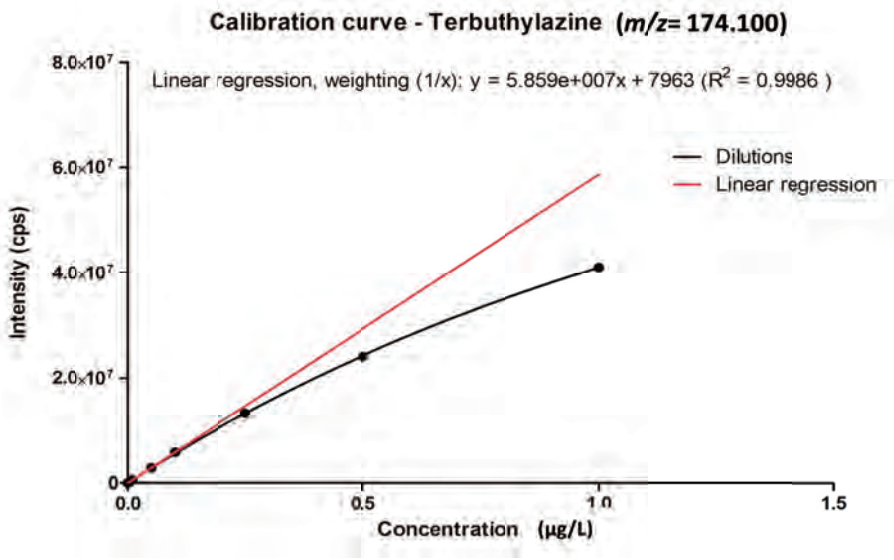


**Figure 7. Determination of TBA ULOQ using linear regression and 1/x weighting**

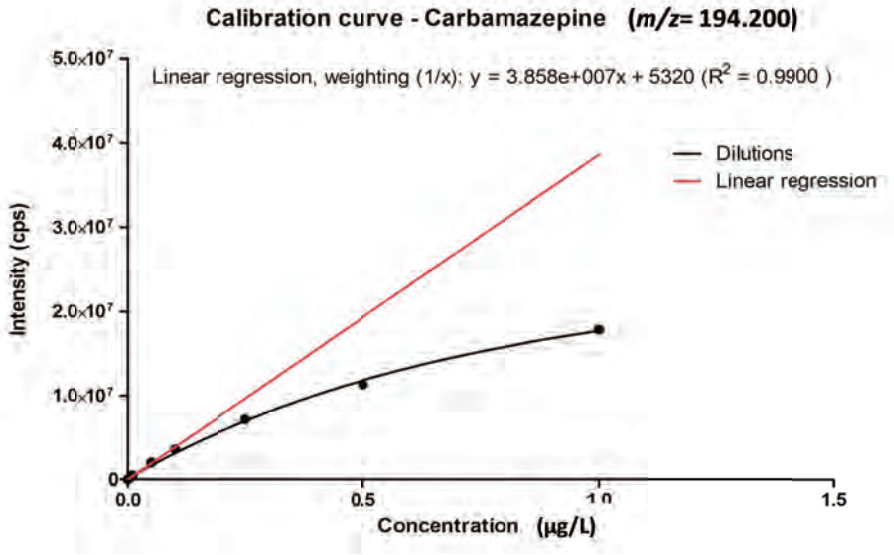


**Figure 8. Determination of CBZ ULOQ using linear regression and 1/x weighting**

## 6.3 Recoveries from SPE cartridges

Having established the LLOQ and ULOQ, we next tested various commercially available SPE cartridges to determine which product gave the best recoveries. The cartridge with the best recoveries for ATR, TBA and CBZ was chosen for further analysis. We defined a minimum acceptable threshold for recoveries to be 40%. Cartridges were purchased from Waters, Supelco and Phenomenex (see Table 7).

**Table 7. Cartridges used for sample capture**

| Cartridge | Supplier | Sorbent Lot | Part number |
|---|---|---|---|
| Strata (200 mg/6 mL) | Phenomenex | S201-96 | 8B-S001-FCH |
| Stata-X 33 µm Polymeric reversed phase (500 mg/6 mL) | Phenomenex | S300-145 | 8B-S100-HCH-S |
| Stata-X 33 µm Polymeric reversed phase (100 mg/6 mL) | Phenomenex | S300-112 | 8B-S100-ECH-S |
| Discovery DSC 18 (500 mg/6 mL) | Supelco | SP2518 | 52606-U |
| Oasis HLB (500 mg/6 mL) | Waters Corporation | 092B31246A | 186000115 |

Samples of 1 ml were prepared in auto-sampler vials, where the 1 ml sample was spiked with 10 µl of a 1 mg/L stock solution, giving a final concentration of 10 ng/L for each compound in separate samples, or 10 ng/L for each compound in a mixture of the three compounds. Similarly, 1 L volumes were also spiked with the same amounts of stock solution. The 1 L volumes of water was passed through the cartridges and eluted, following the manufacturer's recommendation. The percentage yield of each of the ECs concentrated in a cartridge compared to that of the EC in the 1 ml spiked sample was determined in triplicate for each of the three compounds, and for both the two most intense MRM fragments. The recoveries obtained with the five different cartridges are shown in Table 8.

**Table 8. A comparison of the recoveries for ATR, TBA and CBZ using five different SPE cartridges.**

| Cartridge type | Peak area of analyte fragments | | | | | |
|---|---|---|---|---|---|---|
| | ATR 1 | ATR 2 | CBZ 1 | CBZ 2 | TBA 1 | TBA 2 |
| Discovery DSC 18 1 g | 93% | 90% | 36% | 35% | 89% | 88% |
| HLB 500 mg | 93% | 91% | 32% | 31% | 87% | 92% |
| Strata X 500 mg | 103% | 101% | 28% | 27% | 100% | 98% |
| Strata X 100 mg | 82% | 81% | 28% | 27% | 78% | 77% |
| Strata 18 CE 200 mg | 116% | 114% | 44% | 42% | 105% | 104% |

Although all five different cartridges accomplished significant recoveries for ATR and TBA, only the Strata 18 CE 200 mg SPE unit provided a yield in excess of 40% for CBZ. We therefore decided to process all samples in the national reconnaissance study using this cartridge.

## 6.4 Accuracy and precision

Accuracy and precision were tested simultaneously using stock solutions of mixtures at three different concentrations. According to the FDA requirement for the determination of accuracy and precision, the analysis method should at least be tested at the LLOQ, the ULOQ and a value in between the two extremes (mid-value). These concentrations were spiked into three separate 1 L dd$H_2O$ samples. Extraction was performed using the Strata 18-CE 200 mg cartridges. Extractions were performed in triplicate at each concentration, and three replicate analysis runs were also done to test instrument variation. The precision and accuracy were determined from the average of the instrument replicates (Table 9).

**Table 9. Precision and accuracy at LLOQ, mid-range concentration and ULOQ**

LLOQ (ATR 100 ng/L, TBA and CBZ 50 ng/L)

| Sample Name | Peak area of analyte fragment | | | | | |
|---|---|---|---|---|---|---|
| | ATR 1 | ATR 2 | CBZ 1 | CBZ 2 | TBA 1 | TBA 2 |
| LLOQ 1 | 6140 | 2022 | 2103 | 542 | 12555 | 2249 |
| LLOQ 2 | 7896 | 2349 | 2403 | 464 | 13183 | 2361 |
| LLOQ 3 | 7689 | 2428 | 2320 | 489 | 13811 | 2474 |
| LLOQ average | 7242 | 2267 | 2276 | 498 | 13183 | 2361 |
| Precision (Cv) | 13% | 10% | 7% | 8% | 5% | 5% |
| Accuracy (Bias) | 4% | -2% | 3% | -4% | -10% | -7% |

Mid-value (50 µg/L)

| Sample Name | Peak area of analyte fragment | | | | | |
|---|---|---|---|---|---|---|
| | ATR 1 | ATR 2 | CBZ 1 | CBZ 2 | TBA 1 | TBA 2 |
| Mid 1 | 1770000 | 639000 | 874000 | 208500 | 2512500 | 465417 |
| Mid 2 | 2315000 | 840000 | 966500 | 228000 | 3204167 | 586250 |
| Mid 3 | 2265000 | 816000 | 877500 | 208500 | 2962500 | 542083 |
| Mid average | 2116667 | 765000 | 906000 | 215000 | 2893056 | 531250 |
| Precision (Cv) | 14% | 14% | 6% | 5% | 12% | 12% |
| Accuracy (Bias) | 0% | 1% | 7% | 2% | 1% | 3% |

ULOQ (100 µg/L)

| Sample Name | Peak area of analyte fragment | | | | | |
|---|---|---|---|---|---|---|
| | ATR 1 | ATR 2 | CBZ 1 | CBZ 2 | TBA 1 | TBA 2 |
| ULOQ 1 | 4229412 | 1535294 | 1277600 | 305200 | 6035000 | 1127000 |
| ULOQ 2 | 4270588 | 1570588 | 1544000 | 361600 | 6620000 | 1246000 |
| ULOQ 3 | 4352941 | 1501765 | 1640000 | 389600 | 6165000 | 1143500 |
| ULOQ average | 4284314 | 1535882 | 1487200 | 352133 | 6273333 | 1172167 |
| Precision (Cv) | 1% | 2% | 13% | 12% | 5% | 6% |
| Accuracy (Bias) | -6% | -3% | 2% | 1% | -6% | -5% |

It is clear from Table 9 that the precision of the quantitation, expressed as the coefficient of variation, is within the 15% range prescribed by the FDA for bioanalytical method validation for each of ATR, CBZ and TBA (FDA, 2001). The determined biases for all three compounds were consistently less than 10% at each of the three concentration values.

## 6.5 Stability

The stability of diluted as well as concentrated samples was determined at three incubation times. Two samples that contained mixtures of ATR, CBZ and TBA were quantitated at 0 h, 6 h and 24 h. No statistically significant difference between the concentrations of the three compounds at the three different analysis times were detected for either the diluted or concentrated samples, showing that the compounds remained stable at 22°C for up to 24 h.

## 6.6 Sample collection and preservation

Water samples are typically collected in amber bottles to limit exposure of photo-labile compounds to light. Bottles are sometimes salinised with 5% dimethyldichlorosilane to minimise adsorption of analytes to the glass surface. Sample integrity is also maintained by keeping samples on ice after collection. On arrival at the laboratory samples are stored in at 4˚C. Some laboratories have also reported adjusting the sample pH as a preservative precaution. Since different unknown analytes may respond differently to acidic and alkaline conditions, some groups have reported using two samples adjusted to acidic and basic pH values. However, Wulfeck-Kleier *et al*. illustrated that pH had an adverse effect on ATR (2010). We therefore decided not to adjust sample pH, but rather to limit storage times at 4°C between sample collection and analysis.

Samples were collected from eight different locations in South Africa. Samples were therefore collected at points immediately before the purified water entered the reticulation system in Johannesburg, Pretoria, Pietermaritzburg, Durban, Cape Town, Port Elizabeth and Bloemfontein (water treatment plant and tap water). Tap water was also collected from southern Bloemfontein. The collection points are shown in 9. A

prerequisite from some of the water treatments plants before agreeing to participate in this study was that we not identify the source of water samples beyond the metropolitan area where the plant is situated. For this reason, the individual water treatment plants are not uniquely identified in this report. Samples (2 L volumes) were collected from all plants during February, May, August and November of 2012. Samples were typically transported to the laboratory within 24 -48 hours and directly processed for analysis.

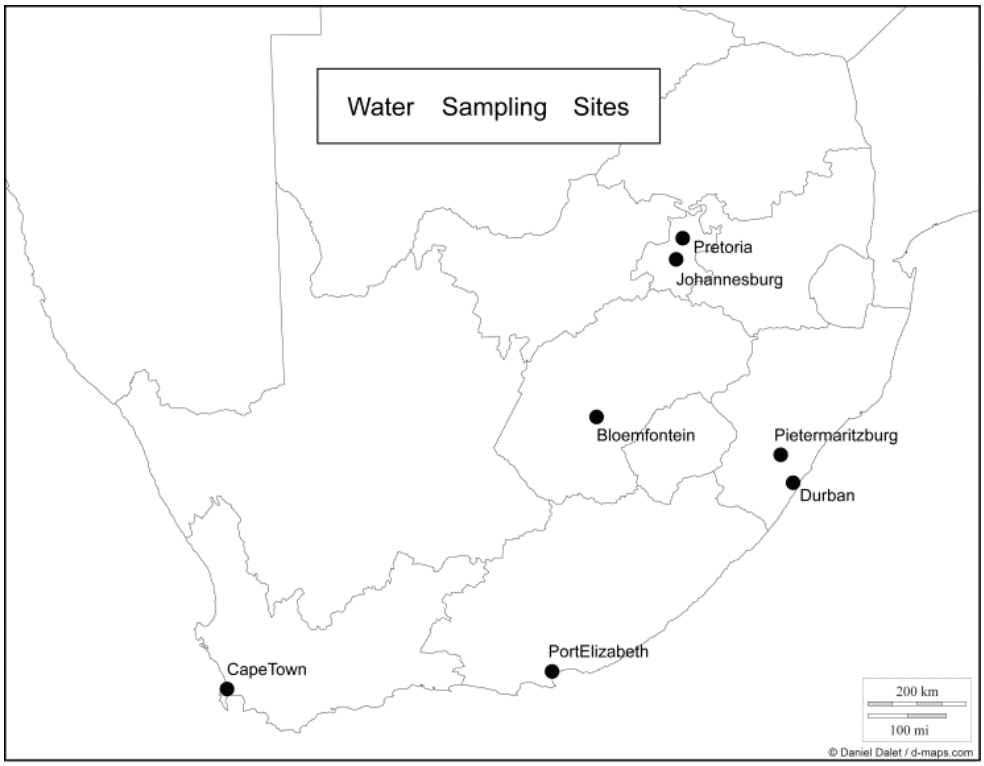


**Figure 9. Geographic distribution of the sampling points used in the national reconnaissance study.**

## 6.7 SPE extraction

The atrazine, terbuthylazine, carbamazepine and deuterated atrazine standards for mass spectrometry, used for quantification, were purchased from Sigma Aldrich. Before extraction sample bottles, cartridges and tubes used for elution were labelled. Extractions were performed using a Supelco Visiprep DL manifold. Each cartridge was prepared by passing through 6 mL MeOH followed by 6 mL of $ddH_2O$. Samples were loaded at an approximate rate of 8 mL/min. Cartridges were then washed with 6 mL of ddH2O and left for 30 min to dry. After 30 min. elution tubes were loaded into the manifold, and cartridges were sequentially eluted with 2 mL of MeOH and 2 mL of ethyl acetate. The eluate was dried *in vacuo* using a Savant SC 210A Speedvac concentrator, Thermo RVT 4104 refrigerated vapour trap and a Thermo OFP 400-230 pump. After extracts were brought to near dryness, the extracts were reconstituted with 1 mL ddH2O / 0.1% FA and suspended using a VELP Scientifica vortex and sonication, where necessary. Samples were then applied to the HPLC-MS.

## 6.8 HPLC MS-MS

We used an Agilent 1200 LC coupled with an ABSciex 3200 QTRAP mass spectrometer. The LC was fitted with a Gemini-NX 3U C18 110Å (150 x 2 mm) column. Samples (injection volume of 20 µL) were resolved at a flow rate of 0.3 mL/min. We used dd$H_2O$ / 0.1% FA (solvent A) and MeOH / 0.1% FA (solvent B) as elution solvent. Detection and quantification of analytes were monitored using MRM with settings for the major transitions of each analyte as is shown in Table 10. During our analysis, two fragments of each precursor analyte were measured (see Table 10). This improved the reliability of the detection as well as the accuracy of quantitation.

**Table 10. Precursor and fragment masses of the three ECs**

| | Precursor mass(Q1) | Fragment 1 (Q3) | Fragment 2 (Q3) |
|---|---|---|---|
| Atrazine | 216.049 | 174.100 | 104.000 |
| Terbuthylazine | 230.033 | 174.100 | 104.000 |
| Carbamazepine | 237.100 | 194.200 | 192.100 |

## 6.9 Continuous quality assurance throughout analysis

Throughout the study certain quality checks were performed to ensure that the instrumentation remained calibrated and maintained good accuracy, traceability, consistency, homogeneity and stability. Since the sampling spans a period of one year to allow detection of seasonal variation, rigorous documentation of quality procedures was followed and recorded.

# 7 Results

## 7.1 Qualitative screening

A combined total of 34 pesticides or pharmaceuticals out of 618 tested were detected in the sourced water samples. The list of pesticides and pharmaceuticals and other ECs that were part of the library, and that we could detect, is given in Appendix D. A list of the detected chemicals was compiled using the application entries of the Hazardous Substances Data Bank (HSDB; See Table 11) (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB). Note that no cyanotoxins were detected in any of the drinking water samples.

**Table 11. Combined list of all chemicals detected during qualitative screens of drinking water samples**

| Analyte | Description |
|---|---|
| Adenine | Adenine is a nucleotide classified as a purine |
| Alachlor | Pesticide |
| Atenolol | Antihypertensive drug for cardiovascular diseases |
| Atrazine | Herbicide |
| Benzocaine | Anaesthetic |
| Carbamazepine | Anticonvulsant / antiepileptic drug |
| Cinchonidine | Antimalarial |
| Cinchonine | Antimalarial |
| Diphenylamine | Fungicide |
| Imazalil | Fungicide |
| Ephedrin | Bronchodilator, increasing airflow to the lungs |
| Flecainide | - |
| Fluconazole | Antifungal |
| Hexazinone | Herbicide |
| Imidacloprid | Insecticide |
| Metazachlor | - |
| Metolachlor | Herbicide |
| Minoxidil | Antihypertensive vasodilator slows or stops hair loss |
| Nalidixicacid | - |
| Nalidixinsaeure | - |
| Oxcarbazepine | Anticonvulsant / antiepileptic drug |
| Paracetamol | An analgesic used as an aspirin substitute |

| | |
|---|---|
| Phenytoin | Antiepileptic drug useful in the treatment of epilepsy |
| Propazine | Herbicide |
| Sebuthylazine-desethyl | Pesticide |
| Simazine | Herbicide |
| Sulfisomidine | - |
| Tebuthiuron | Herbicide |
| Telmisartan | Antihypertensive |
| Temazepam | - |
| Terbumeton | - |
| Terbuthylazine | Herbicide |
| Thiabendazole | Fungicide |

The seasonal fluctuation in the qualitative screening results for each of the sampling sites is given in Tables 12-20 below. Contaminants that were identified in 3 or 4 seasons of the year may be relevant to future epidemiological studies. The last column of each Table shows the cumulative seasonal occurrence of each identified contaminant. Critical contaminants are highlighted in each of the Tables.

Interestingly, an inspection of the screening results throughout the year for all the sites showed that atrazine, terbuthylazine and carbamazepine, chosen as the three predominant ECs, indeed were within the top 4 most frequent contaminants, and were detected 25, 33 and 28 times, respectively.

**Table 12. Contaminants detected in the qualitative screen of drinking water sampled from a water treatment plant in Cape Town**

| Analytes | Summer | Autumn | Winter | Spring | Annual occurences |
|---|---|---|---|---|---|
| Cinchonidine | x | x | x | | 3 |
| Cinchonine | | | | x | 1 |
| Diphenylamine | | | | x | 1 |
| Sulfisomidine | x | | | | 1 |
| **Terbuthylazine** | x | x | x | x | 4 |

**Table 13. Contaminants detected in the qualitative screen of drinking water sampled from a water treatment plant in Port Elizabeth**

| Analytes | Summer | Autumn | Winter | Spring | Annual occurences |
|---|---|---|---|---|---|
| **Atrazine** | x | | | | 1 |
| **Carbamazepine** | | | | x | 1 |
| Cinchonidine | x | x | x | | 3 |
| Diphenylamine | | | | x | 1 |
| Imidacloprid | | | | x | 1 |
| Paracetamol | | x | | | 1 |
| Sulfisomidine | | x | | | 1 |
| Telmisartan | | x | | | 1 |
| **Terbuthylazine** | | | x | x | 2 |

**Table 14. Contaminants detected in the qualitative screen of drinking water sampled from a water treatment plant in Durban**

| Analytes | Summer | Autumn | Winter | Spring | Annual occurences |
|---|---|---|---|---|---|
| **Atrazine** | x | x | | x | 3 |
| **Carbamazepine** | x | x | x | x | 4 |
| Cinchonidine | x | x | x | x | 4 |
| Diphenylamine | | x | | x | 2 |
| Hexazinone | x | x | x | x | 4 |
| Metolachlor | x | | | | 1 |
| Phenytoin | x | x | x | x | 4 |
| Sulfisomidine | | x | | x | 2 |
| Tebuthiuron | x | x | x | x | 4 |
| Telmisartan | x | x | | | 2 |
| **Terbuthylazine** | x | x | x | x | 4 |

**Table 15. Contaminants detected in the qualitative screen of drinking water sampled from a water treatment plant in Pietermaritzburg**

| Analytes | Summer | Autumn | Winter | Spring | Annual occurences |
|---|---|---|---|---|---|
| **Atrazine** | x | x | x | x | 4 |
| **Carbamazepine** | x | x | x | x | 4 |
| Cinchonidine | x | x | x | x | 4 |
| Diphenylamine | x | | | x | 2 |
| Metazachlor | | x | | | 1 |
| Metolachlor | x | | | | 1 |
| Tebuthiuron | x | | x | | 2 |
| Telmisartan | | x | | | 1 |
| **Terbuthylazine** | x | x | x | x | 4 |

**Table 16. Contaminants detected in the qualitative screen of drinking water sampled from a water treatment plant in Johannesburg**

| Analytes | Summer | Autumn | Winter | Spring | Annual occurences |
|---|---|---|---|---|---|
| **Atrazine** | x | x | x | x | 4 |
| **Carbamazepine** | x | x | x | x | 4 |
| Cinchonidine | x | | x | x | 3 |
| Diphenylamine | | | | x | 1 |
| Enilconazole | | x | | | 1 |
| Ephedrin | | x | | | 1 |
| Flecainide | | x | | | 1 |
| Fluconazole | | x | | | 1 |
| Metolachlor | x | | | | 1 |
| Phenytoin | | x | | | 1 |
| Sebuthylazine-desethyl | x | | | | 1 |
| Simazine | | x | | | 1 |
| Sulfisomidine | x | | | | 1 |
| Tebuthiuron | x | x | x | x | 4 |
| Telmisartan | | | | x | 1 |
| Temazepam | | x | | | 1 |
| **Terbuthylazine** | x | x | x | x | 4 |
| Thiabendazole | | x | | | 1 |

**Table 17. Contaminants detected in the qualitative screen of drinking water sampled from a water treatment plant in Pretoria**

| Analytes | Summer | Autumn | Winter | Spring | Annual occurences |
|---|---|---|---|---|---|
| **Atrazine** | x | x | | | 2 |
| **Carbamazepine** | x | x | | x | 3 |
| Cinchonidine | | x | x | x | 3 |
| Diphenylamine | | x | | x | 2 |
| Fluconazole | | x | | | 1 |
| Metolachlor | x | | | | 1 |
| Minoxidil | | x | | | 1 |
| Phenytoin | | x | | x | 2 |
| Tebuthiuron | x | x | | | 2 |
| Telmisartan | | x | | | 1 |
| Terbumeton | | x | | | 1 |
| **Terbuthylazine** | x | x | | x | 3 |

**Table 18. Contaminants detected in the qualitative screen of drinking water sampled from a water treatment plant in Bloemfontein**

| Analytes | Summer | Autumn | Winter | Spring | Annual occurences |
|---|---|---|---|---|---|
| 2-deoxyguanosine | | | x | | 1 |
| **Atrazine** | x | x | | x | 3 |
| Benzocaine | | | | x | 1 |
| **Carbamazepine** | x | x | x | x | 4 |
| Cinchonidine | x | x | x | x | 4 |
| Diphenylamine | | x | | x | 2 |
| Ephedrin | | | x | | 1 |
| Fluconazole | x | | x | x | 3 |
| Metolachlor | x | | | | 1 |
| Nalidixicacid | | | x | | 1 |
| Nalidixinsaeure | | | x | | 1 |
| Phenytoin | x | x | x | x | 4 |
| Tebuthiuron | x | x | x | x | 4 |
| Telmisartan | | x | | x | 2 |
| **Terbuthylazine** | x | x | x | x | 4 |

**Table 19. Contaminants detected in the qualitative screen of tap water sampled from a northern Bloemfontein residential area**

| Analytes | Summer | Autumn | Winter | Spring | Annual occurences |
|---|---|---|---|---|---|
| **Atrazine** | x | x | x | x | 4 |
| Benzocaine | | | x | | 1 |
| **Carbamazepine** | x | x | x | x | 4 |
| Cinchonidine | x | x | x | x | 4 |
| Diphenylamine | | | | x | 1 |
| Fluconazole | x | | x | x | 3 |
| Metolachlor | x | | | | 1 |
| Phenytoin | x | x | x | x | 4 |
| Tebuthiuron | x | x | x | x | 4 |
| Telmisartan | | | | x | 1 |
| **Terbuthylazine** | x | x | x | x | 4 |

**Table 20. Contaminants detected in the qualitative screen of tap water samples from a southern Bloemfontein residential area**

| Analytes | Summer | Autumn | Winter | Spring | Annual occurences |
|---|---|---|---|---|---|
| Adenine | | | | x | 1 |
| Alachlor | x | | | | 1 |
| Atenolol | | | x | | 1 |
| **Atrazine** | x | x | x | x | 4 |
| Benzocaine | | | x | | 1 |
| **Carbamazepine** | x | x | x | x | 4 |
| Cinchonidine | | x | x | x | 3 |
| Diphenylamine | x | | | | 1 |
| Oxcarbazepine | | | x | | 1 |
| Phenytoin | | | | x | 1 |
| Propazine | x | | | x | 2 |
| Tebuthiuron | x | x | x | x | 4 |
| Telmisartan | | x | | x | 2 |
| **Terbuthylazine** | x | x | x | x | 4 |

## 7.2 Quantitation

The absolute level of carbamazepine, atrazine and terbuthylazine was also quantitated in samples recovered from each of the sampling sites. The results are listed in Tables 21-23 and shown graphically in Figures 10-12.

**Table 21. The concentration (µg/L) of atrazine (ATR) sampled over four seasons. Average concentrations are indicated (n=3).**

| Sampling sites | Summer | Autumn | Winter | Spring |
|---|---|---|---|---|
| BFN North tap | 0.02 | 0.15 | 0.01 | 0.02 |
| BFN South tap | 0.01 | 0.19 | 0.02 | 0.15 |
| Bloemfontein | 0.02 | 0.02 | 0.01 | 0.01 |
| Cape Town | 0.00 | 0.00 | 0.00 | 0.00 |
| Durban | 0.02 | 0.02 | 0.00 | 0.01 |
| Johannesburg | 0.13 | 0.15 | 0.11 | 0.12 |
| Pietermaritzburg | 0.02 | 0.02 | 0.02 | 0.02 |
| Port Elizabeth | 0.01 | 0.00 | 0.00 | 0.00 |
| Pretoria | 0.04 | 0.16 | 0.00 | 0.01 |

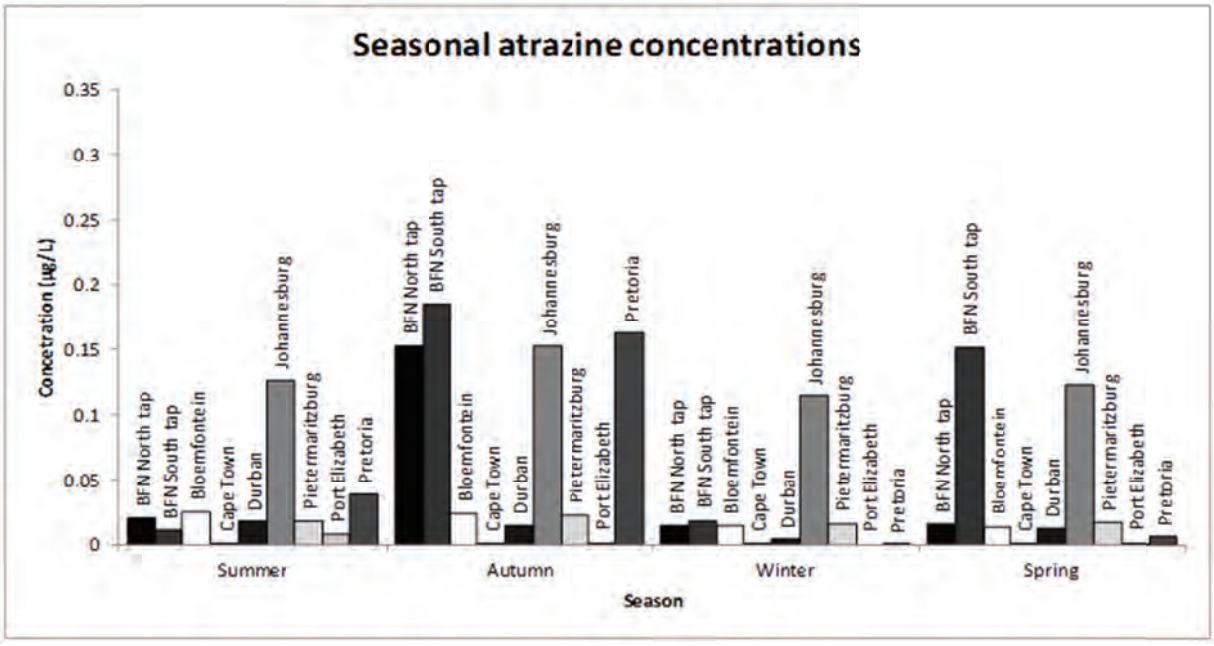


**Figure 10. Quantitation of atrazine in drinking water sampled from water purification plants supplying water to major metropolitan areas in South Africa.**

Atrazine is primarily a weedicide used in sugarcane, corn and sorghum crop production. It would thus be expected that atrazine concentrations should be at their highest during spring and summer, directly after agricultural applications. Interestingly, our results revealed the highest concentration of atrazine in drinking water during autumn. Atrazine was specifically more prevalent in Bloemfontein and Johannesburg, where run-off from farms in major summer grain producing regions could conceivably introduce the weedicide into the drinking water system.

**Table 22. The concentrations (µg/L) of carbamazepine (CBZ) sampled over four seasons. Average concentrations are indicated (n=3).**

| Sampling site | Summer | Autumn | Winter | Spring |
|---|---|---|---|---|
| BFN North tap | 0.30 | 0.04 | 0.16 | 0.15 |
| BFN South tap | 0.02 | 0.02 | 0.02 | 0.04 |
| Bloemfontein | 0.32 | 0.12 | 0.16 | 0.15 |
| Cape Town | 0.00 | 0.00 | 0.00 | 0.00 |
| Durban | 0.01 | 0.02 | 0.00 | 0.01 |
| Johannesburg | 0.01 | 0.01 | 0.01 | 0.01 |
| Pietermaritzburg | 0.00 | 0.00 | 0.00 | 0.00 |
| Port Elizabeth | 0.00 | 0.00 | 0.00 | 0.00 |
| Pretoria | 0.03 | 0.14 | 0.00 | 0.00 |

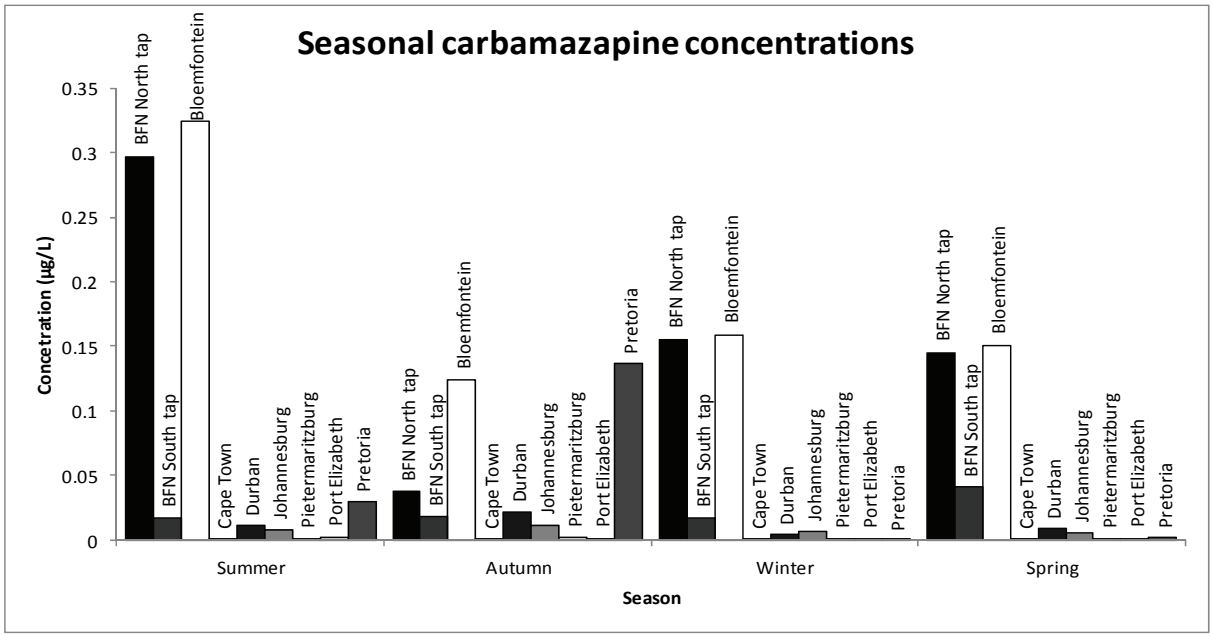


**Figure 11. Quantitation of carbamazepine in drinking water sampled from water purification plants supplying water to major metropolitan areas in South Africa.**

Carbamazapine is an anticonvulsant and mood-stabilising drug, typically used to treat epilepsy and bipolar disorder. Carbamazepine is primarily found at higher concentrations in the tap water of Bloemfontein and Pretoria. In Bloemfontein carbamazepine were at its highest concentration during summer, while in Pretoria concentrations of atrazine increased during autumn.

**Table 23. The concentration (µg/L) of terbuthylazine (TBA) sampled over four seasons. Average concentrations are indicated (n=3).**

| Sampling sites | Summer | Autumn | Winter | Spring |
|---|---|---|---|---|
| BFN North tap | 0.04 | 0.03 | 0.02 | 0.04 |
| BFN South tap | 0.01 | 0.04 | 0.01 | 0.03 |
| Bloemfontein | 0.06 | 0.04 | 0.02 | 0.04 |
| Cape Town | 0.00 | 0.00 | 0.00 | 0.00 |
| Durban | 0.04 | 0.04 | 0.01 | 0.03 |
| Johannesburg | 0.14 | 0.14 | 0.10 | 0.11 |
| Pietermaritzburg | 0.02 | 0.03 | 0.02 | 0.02 |
| Port Elizabeth | 0.00 | 0.00 | 0.00 | 0.01 |
| Pretoria | 0.07 | 0.21 | 0.00 | 0.01 |

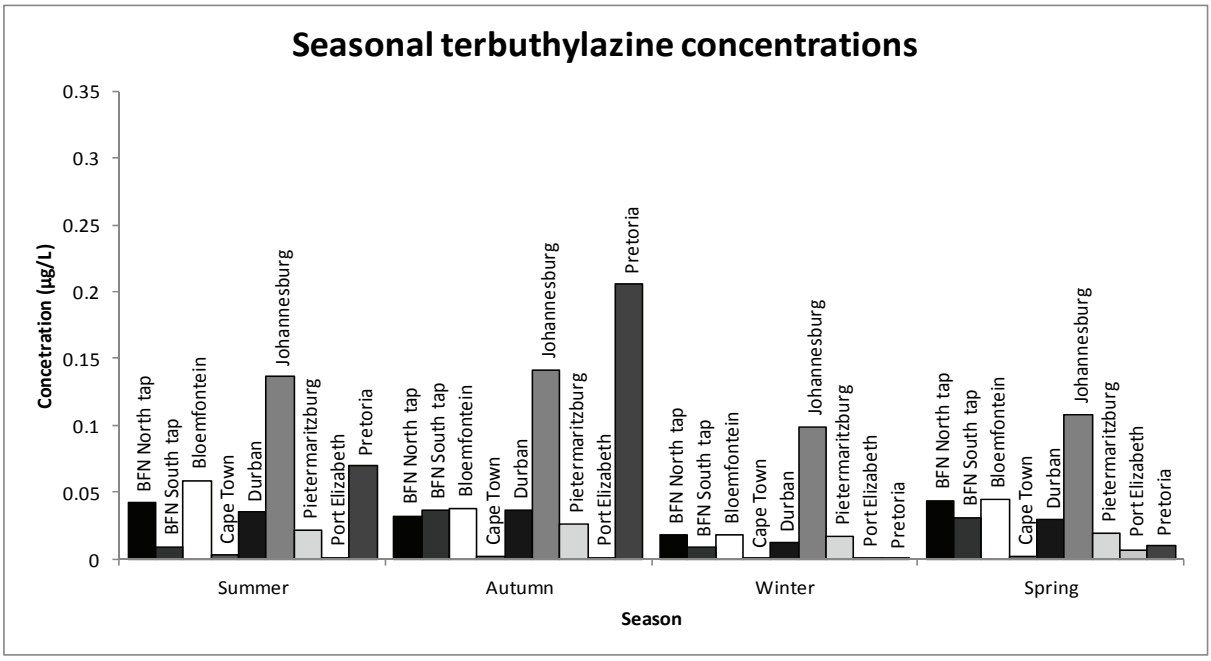


**Figure 12. Quantitation of terbuthylazine in drinking water sampled from water purification plants supplying water to major metropolitan areas in South Africa.**

Terbuthylazine showed a trend in seasonal fluctuation similar to that of atrazine. Concentration levels in Johannesburg displayed little change throughout the year. Terbuthylazine seemed to be used less often compared to atrazine in the water catchment areas feeding into the Bloemfontein water supply. The highest concentrations of terbuthylazine were found in Pretoria during autumn.

### 7.2.1 Maximum Contaminant Levels

The level of contaminants in drinking water is typically interpreted in terms of a threshold or maximum limit concentration at which deleterious health effects are not observed. The EPA has defined such a level, called the maximum contaminant level (MCL).

These levels for the three respective contaminants quantitated above are:

Atrazine MCL= 3 µg/L

Terbuthylazine MCL = 3 µg/L

Carbamazepine MCL = 12 µg/L

In our analysis, *all* of the concentrations determined were *below* this limit for the samples from all the water treatment plants in each of the four seasons.

## 7.3 Risk matrix

Commonly total health risk is determined by the hazard, vulnerability to the hazard and people's capacity to overcome the hazard. The formulation of hazard is shown in equation 4.

$$Risk = Hazard \times \frac{vulnerability}{capacity\ to\ overcome} \quad \text{(Eq. 4)}$$

A short discussion of each of these factors is given below.

### 7.3.1 Hazard

A risk hazard matrix can be constructed according to the severity of the hazard and the frequency of the hazard occurring. In order to scale severity in a range from 1 to 5, where 5 represent extremely severe cases and 1 represent negligible cases, hazardous effects obtained from the Hazardous Substance Database (HSDB) were categorised as shown below.

#### *7.3.1.1 Atrazine*

Ingestion of 100 g of atrazine was observed to have catastrophic results. According to literature such exposure may lead to coma, heart and peripheral vessel damage, and renal failure, resulting in death (Ellenhorn et al., 1997a; Gerberding 2003). Other delayed, fatal consequences include leukaemia and brain cancer (Bingham et al., 2001b; Gerberding 2003).

Exposure to atrazine also had other critical carcinogenic risks. Tumours of the reproductive organs were often related to atrazine exposure (Bingham et al., 2001b; Gerberding 2003). Several studies on lymph cancer also showed an association with atrazine exposure. Alarmingly, one study by Van Leeuwen *et al.* (1999b) revealed an association between stomach cancer and atrazine exposure in the range 50-649 ng/L, which is well below the EPA's MCL of 3 µg/L for drinking water. Other critical effects revolve around vulnerabilities of women during pregnancy and on the foetus or infants. These effects include foetal development aberrations like growth retardation, a decrease in gestation age, and gastroschisis (Chevrier et al., 2011; Munger et al., 1997a; Ochoa-Acuna et al., 2009; Waller et al., 2010). Lower birth weight was also directly associated with perinatal mortality in the US (Paneth, 1995).

Moderate effects of exposure include a decrease in sperm concentration and motility, known as oligospermia (Swan, 2006).

Minor side effects of atrazine exposure included fatigue, dizziness, nausea, and skin irritation (Ellenhorn et al., 1997a; Gerberding 2003).

#### *7.3.1.2 Terbuthylazine*

The HSDB has very little information on the effects of terbuthylazine exposure on health, although some of the effects are shared with other triazine-based chemicals. Therefore terbuthylazine shares most of the side effects caused by over-exposure to atrazine. A critical characteristic of terbuthylazine is its carcinogenic properties. Triazine ring compounds are typically linked to leukaemia, brain cancer and cancer of reproductive organs (Gerberding 2003).

Minor side effects of terbuthylazine are mild skin and eye irritations (Hogg et al., 1981; USEPA).

#### *7.3.1.3 Carbamazepine*

Catastrophic effects of exposure to carbamazepine include prolonged epileptic episodes. According to these studies (Lifshitz et al., 2000; McEvoy, 2007) prolonged epileptic crises may result in death. Other catastrophic effects include lupus erythematous syndrome, agranulocytosis and aplastic anemia, which may result in death (Lifshitz et al., 2000; McEvoy, 2007).

Critical effects of carbamazepine overdose include impacts on vital organ like cardio toxicity and myocarditis, which may lead to a weakened heart and ultimate heart failure. It also includes renal failure, lymphatic diseases and infant developmental restrictions, like reduced gestational age, cleft palate, spina bifida and reduced cognitive development (Hogg et al., 1981; Matalon et al., 2002; Scolnik et al., 1994; Takamiya et al., 2006; Todorovic et al., 1993).

Some moderate effects were also identified in the literature. Hayashi *et al.* investigated the relation between carbamazepine and sperm count and motility. In their case study a man taking a dose of 400 mg of carbamazepine daily for 13 years showed very low sperm counts with almost no motility (2005). Once his medication changed these levels recovered significantly.

Nystagmus and drowsiness are two of the effects of carbamazepine overdose that varies according to the degree of exposure. Minor nystagmus cases result in blurred vision due to involuntary movement of the eyes, while more severe cases may result in vertigo, nausea and dizziness. Overall the severity of this is minor in comparison to other effects of overdose, since these are temporary, and presumably reversible, symptoms. One study reported that carbamazepine serum concentrations of 18 µg/mL to 32 µg/mL led to nystagmus in children (Lifshitz et al., 2000).

#### *7.3.1.4 Categorising hazard*

Knowing the effects of exposure to different levels of atrazine, terbuthylazine and carbamazepine allows one to scale these effects according to severity. Unfortunately, most clinical cases of over-exposure to contaminants are isolated incidences; and it is often difficult to reliably relate the exact concentration to a specific effect. Also, care must be taken to establish that there is a single modal relationship between contaminant exposure and health effects. In some cases attempts have been made to determine the

contaminant exposure through measurement of serum concentrations, which provides some indication of exposure, but is largely regarded as unreliable. The information gathered from the HSDB contained very sparse information relating exposure to health effect, which makes the determination of hazard risk very challenging. Importantly, each contaminant will have a unique hazard risk matrix, depending on dosage levels, solubility and half-life of contaminants.

In addition to these difficulties, there is the question of reliability of information. A case study mentioned above reported a link between atrazine levels in the range 0.05-0.649 µg/L and stomach cancer. The concentration reported in that single study is well below the EPA's MCL of 3 µg/L (Van Leeuwen et al., 1999b). To be able to construct a hazard risk matrix, some assumptions have to be made. Assuming that the MCL proposed by the EPA is reliable, this implies that exposure to an atrazine level of less than 3 µg/L will cause negligible hazard risk. As such the study of Van Leeuwen *et al.* (1999b) should then be disregarded.

According to the EPA's toxicological analyses of the exposure of fish to atrazine, toxicity levels can be categorised in the following ranges: acute and catastrophic cases occurred at concentrations in excess of 5300 µg/L, and moderate effects like reduction in reproduction rate occurred at 62-88 µg/L. Defining the category "serious" to lie between "catastrophic" and "moderate", means that serious effects will occur in the concentrations range of 88-5300 µg/L. Finally, minor side-effects like fatigue, dizziness, nausea occurred at in the concentrations range of 5-20 µg/L, while concentrations below 5 µg/L had negligible effects (http://www.epa.gov/oppsrrd1/REDs/atrazine_combined_docs.pdf). If, as a first approximation, it is assumed that these levels are also valid for human hazard risk, the categories of severity of atrazine exposure in humans can be divided as shown in Table 24.

**Table 24. Approximate concentrations for different severity categories.**

| Category | Concentration (µg/L) |
|---|---|
| Negligible | < 5 |
| Minor | 5 - 20 |
| Moderate | 20 - 88 |
| Serious | 88 - 5300 |
| Catastrophic | > 5300 |

As mentioned above, severity is then scaled in a range from 1 to 5 (see Table 25).

**Table 25. Scale of severity of consequences of contaminants**

| Description | Category / score | Health results criteria |
|---|---|---|
| Negligible | 1 | Impact unnoticable |
| Minor | 2 | Temporary disability |
| Moderate | 3 | Non-fatal prolonged disability |
| Serious | 4 | Permanent major disability, may result in death |
| Catastrophic | 5 | Most likely resulting in death |

Once severity has been defined, the seasonal frequency may be used to develop a hazard-based risk matrix. The frequency scoring table is shown in Table 26.

**Table 26. Frequency of seasonal detection**

| Decription | Category / Score | Frequency of incident |
|---|---|---|
| Only one season | 1 | 1 |
| Two seasons a year | 2 | 2 |
| Three seasons a year | 3 | 3 |
| The whole year | 4 | 4 |

Frequency of contaminant levels reaching hazardous concentrations can also be calculated using quantitative results. Hazardous concentration can be determined by levels specified in the literature or the MCL levels specified by the EPA. The integration of the severity and the frequency of a hazardous event is then used to construct a hazard risk scoring matrix, as is shown in Table 27.

**Table 27. Hazard risk classification and scoring through integration of hazard severity and frequency parameters**

| Frequency | Severity | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| 4 | 4 | 8 | 12 | 16 | 20 |
| 3 | 3 | 6 | 9 | 12 | 15 |
| 2 | 2 | 4 | 6 | 8 | 10 |
| 1 | 1 | 2 | 3 | 4 | 5 |

According to this scoring scheme, hazard risk may then be divided into regions that are categorised as low (1-2), medium (3-6), high (8-12) and extreme (15-20) risks, as is shown by the coloured blocks in Table 27. Referring back to our quantitative data in Table 21, it is clear that overall severity of hazard is negligible and that occurrence per year will primarily determine the hazard risk. Accordingly, we propose, as a first approximation, that metropolitan areas with an occurrence greater than 2 seasons a year indicate a medium hazard risk, while 2 or fewer seasonal occurrences per year indicate low hazard risks. This means that in the case of atrazine, samples taken from domestic taps in both northern and southern Bloemfontein, as well as the water purification plants in southern Bloemfontein, Johannesburg, Pietermaritzburg and Durban represented medium hazard risk. Correspondingly, Pretoria, Port Elizabeth and Cape Town had a low hazard risk due to atrazine exposure.

Since terbuthylazine share many properties with atrazine, it is likely that its categories will be similar, meaning that the drinking water in Bloemfontein, Johannesburg, Pietermaritzburg and Durban represented medium risk, and the remainder of the metropolitan areas tested represented low risk.

Carbamazepine was also routinely detected at levels well below the MCL of 12 µg/L as stipulated by the EPA. Assuming a severity score of 1 at this level, the resultant hazard risk due to carbamazepine exposure would be low in Cape Town and Port Elizabeth where it was not detected or detected in only one season, and medium in other metropolitan areas, where it was detected twice or more during the year.

The central idea is that each contaminant will have its own, unique hazard risk matrix. Ideally, the exposure of a population to contaminants should be routinely assessed and scored using a hazard risk matrix, which, itself, should be actively maintained and updated with any new scientific data. The continual assessment of exposure to contaminants will enable the identification of trends that may reveal a substantive increase or decrease of specific contaminants in drinking water, and associated hazard risks.

In order to determine total risk of a population, it is crucial to also investigate vulnerability and capacity to overcome hazard posed to a specific community. This is discussed below.

### 7.3.2 Vulnerability

Vulnerabilities are commonly divided according to economic, environmental and social factors. Although ECs impact on each of these factors, vulnerability is primarily determined by the social impact of the contaminant that may influence economic or environmental factors. Investigation of the hazards posed by the three critical ECs revealed that pregnant women, infants, children and the elderly are more susceptible to hazardous effects caused by these contaminants. Vulnerability of communities could therefore be determined by population demographics and economic level.

Typically, individuals in the financially affluent level are characterised by lower reproductive rates, lower infant mortalities, and better developed infrastructure, medical support and water supply. Individuals in the financially constrained level are generally restricted to rural and informal settlements, characterised by poor infrastructure contributing to poor living conditions, and higher reproductive rates. Our study focused on drinking water from water treatment plants in metropolitan cities of South Africa where impacts may be pronounced due to the exposure of large population groups. However, our hazard risk matrix highlights the importance of a closed study system, where people affected by contaminants are defined very precisely and carefully. Essentially people that may be impacted the most negatively by water contaminants were not included in this study. This should be addressed in a further study.

### 7.3.3 Capacity to overcome hazard

The capacity of a population to overcome a hazard is determined by its ability to avoid, mitigate or remedy a hazard. This implies continuous infrastructural development and improvement. Infrastructural development includes water supply networks, education, health services and the overall improvement of the quality of life of a population. In the context of ECs, it requires the introduction of methods to regularly monitor the presence and concentration of ECs in drinking water. Without procedures to monitor ECs, concentration levels cannot be quantitated and thus enforced, and therefore the risk created cannot be avoided, mitigated or remedied.

The National Water Act gazetted in 1998 focused on the management and protection of water quality (1998b). According to section 1 of this act, national government, as public trustee of South Africa's resources, must ensure that water is protected, used, developed, conserved, managed and controlled in a sustainable and equitable manner for the benefit for all persons and in accordance to its constitutional mandate.

The responsibility of ensuring clean drinking water does not depend on government alone. According to section 28 of the National Environmental Management Act, every person who causes, has caused or may cause significant pollution or environmental degradation, must take reasonable measures to prevent such pollution or degradation from occurring, continuing or re-occurring(1998a). This directly forces manufacturers to take counter measures for any pollution that their products may cause or have caused.

However, the primary responsibility still remains with government, since the threat of ECs may not necessarily be noticed if they are not considered as a causal factor (without monitoring). In the light of this study, we propose that selected metropolitan and rural areas are regularly screened for the presence of water soluble pesticides, pharmaceuticals, and personal care products. Individual contaminants that were shown in peer-reviewed literature to cause moderate, serious or catastrophic effects in particular, should then be quantitated, if detected. Health hazard matrices should be constructed for each of these higher risk contaminants where the medical or epidemiological data are available, and these matrices should be maintained and kept current with new developments. The hazard risk of quantitated contaminants should be derived from the constructed matrices. This should be part of a normal, legislatively-enforced water quality assessment programme, and should be an expansion of the SANS241 specification.

# 8 Knowledge Gaps and Future Research

## 8.1 Knowledge and Skill Gaps

There is no enforced testing scheme in the current SANS241-2011 specification to establish the presence or determine the level of pesticides and pharmaceuticals that are regarded as ECs. For this reason, a working knowledge of ECs and methods required to detect and quantitate ECs is poorly developed in the wider water treatment community. There is a significant absence of knowledge on what constitutes an EC, or the techniques that are used and instrument sensitivities in assessing and quantitating ECs. There is also limited knowledge on the possible, diverse health impacts of ECs.

There is therefore a real need for a focused, short training course on ECs in drinking water, an overview of the programmes that have been established internationally to monitor and define ECs, as well as an introduction to modern LC-MS/MS techniques that are used to survey and quantitate ECs. This is an issue that universities should give attention to, particularly with our keen appreciation of the critical importance of correct water management, and the establishment of research capacity in this theme. Specialised post-graduate diploma courses that train candidates in modern water purification techniques, analysis, and the management of water quality is becoming increasingly necessary.

Importantly, in the light of the findings of this study, the establishment of EC testing abilities at individual purification plants currently appear unnecessary. It should also be noted that LC-MS/MS instruments are expensive, and that the correct and scientifically rigorous application of the LC-MS/MS technique requires advanced skills and training. Although suitable training facilities are available at some universities nationally, it is reasonable to suggest that when the technique is needed in the water research and treatment community in South Africa, collaborations or service agreements should be set up with facilities where this technique is well established.

## 8.2 Future Research

This study addressed the presence and levels of three ECs in purified drinking water. It also demonstrated the presence of many other ECs which, although they were present less frequently in the sampled water, have been associated with serious health effects such as leukaemia. There is currently no information on the concentrations of such less-frequently observed ECs in drinking water. It is therefore prudent to continue with the periodic screening of an expanded range of water samples, and to focus quantitation of an EC where the frequency of observation increases, or where serious health effects are associated with a specific compound. Such a screening programme should be implemented at a national scale, and include water purification plants from smaller towns. Do we have the capacity and skills required for such an expanded programme? In this study, the quantitation of a compound typically required an LC/MS-MS run of approximately 20 min. It is therefore theoretically possible to perform about 72 runs per day. Given that the method involved MRM, it is possible to perform many quantitations in a single instrument run. It should therefore be possible to perform several hundred quantitations per week. It is therefore clear that the UFS has the capacity to undertake an expanded programme that involves the periodic screening and quantitation

of ECs in drinking water samples. Such an expanded study would provide better information for the development of guideline values and risk matrices, where appropriate.

Furthermore, there is currently no information on the presence of ECs in surface and groundwater. The proximity of surface or groundwater to sites of intense agricultural activity or land-fill and medical waste dumping sites may cause the presence of very high levels of ECs in such water sources. The domestic use of water retrieved from rivers, dams and boreholes, which is often the norm in rural and farming communities, may expose humans to levels of ECs significantly higher than that detected in the purified water that is supplied to metropolitan areas. This may expose rural communities to high health risks. This is an issue that should be studied, and, if necessary, point-of-use technologies tested that allow the cheap and efficient removal of high risk ECs from drinking water.

## 8.3 Proposed Terms of Reference for a Future Research Project

### 8.3.1 Rationale

Little information is available on the presence of ECs in non-purified water, such as water in dams, rivers or bole-holes. Rural communities, who typically retrieve water from such sources for domestic use, may be at risk of exposure to ECs from these water sources. The ECs present in such water sources could come from run-off of pesticides used in the agricultural industries, chemical waste products from the manufacturing industries, run-off and seepage from landfill sites, including seepage from medical waste dumping sites. The levels of ECs in non-purified water may thus be high, since this water has not been subjected to any standard purification procedure. The prolonged exposure of humans to these ECs by consumption on non-purified water may therefore involve a significant health risk.

### 8.3.2 Objectives

#### *8.3.2.1 General*

The aim of this proposed research is to establish the level of ECs in untreated drinking water that is sourced from rivers, dams and boreholes. Water from these sources may contain elevated levels of ECs, particularly when adjacent to areas of intense agricultural activity or land-fills and medical waste dumping sites. Seepage from medical waste sites may also enter the groundwater system, and thus appear in borehole water. Since this water is not subjected to any formal purification scheme, it is possible that the concentration of ECs are high, and that the human consumption of such water could involve significant health risks to humans. The aim of this project is to provide information on whether the use of non-purified water in rural communities involves a significant health risk to the population.

#### *8.3.2.2 Specific*

- Identification of suitable collection sites. This could include sites in rivers and dams and boreholes where high levels of EC are expected due to proximity to intensive agricultural land-use, discarding of industrial chemical waste, or the presence of land-fill or medical waste dumping sites.

- Assessment of the consumption by rural communities. This may include both observation and questionnaires to establish whether an identified site is the only source of water available to the community for consumption.
- Comprehensive qualitative survey of ECs present in water sourced from the identified sites, and identification of ECs that may pose a significant health risk due to occurrence frequently and health impact.
- Quantitation of identified high risk ECs. This will likely involve LC-MS/MS and the use of a multiple reaction monitoring method.
- Assessment of the possible health impact of the identified putative high risk ECs. This could involve a review of the established medical literature on the health effect of the ECs.
- Study and testing of cheap methods that could be used by the local community to remove high risk ECs from drinking water.

# 9 Conclusions

- A cumulative total of 34 pharmaceuticals or pesticides were detected in these water samples, demonstrating that a wide range of pharmaceuticals and pesticides were present in South African drinking water.
- The herbicides atrazine and terbuthylazine were consistently present at elevated levels (relative to the other samples) in Johannesburg. The anticonvulsant and mood-stabilising drug, carbamazepine, was consistently present at elevated levels (compared to the other samples) in Bloemfontein.
- The highest detected levels of atrazine, terbuthylazine and carbamazepine never exceeded 10% of the Maximum Contaminant Level stipulated by the Environmental Protection Agency in the Unites States, a level generally regarded as safe, were no adverse medical affects are likely to be observed.
- A hazard risk matrix was developed as a first approximation. Although the hazard severity of all three quantitated ECs were suggested to be negligible, the frequency of occurrence suggested the following hazard risks:
    - Atrazine posed a medium risk in the drinking water in Bloemfontein, Johannesburg, Durban and Pietermaritzburg.
    - Terbuthylazine posed a medium risk in the drinking water from all 7 sourced metropolitan areas.
    - Carbamazepine posed a medium risk in the drinking water of Pretoria, Johannesburg, Bloemfontein, Durban, and Pietermaritzburg.

# 10 Recommendations

1. The frequency and level of detected ECs reported in this study do not require any specific and aggressive remedial action at this time.

2. Although the quantitated levels of the three most frequently-observed ECs were less than 10% of the MCL, the range of ECs observed may indicate a growing problem. A national programme in which drinking water is seasonally or bi-annually qualitatively screened, and frequently-observed ECs are quantitated, should be considered.

3. The proposed hazard risk matrix showed that we lack information on the vulnerability of populations and their capacity to overcome the posed hazard. This ability is particularly acute for economically repressed, rural populations that were excluded by the scope of this study. It is recommended that a similar qualitative screen and quantitation of the level of selected ECs be undertaken in one or more rural communities that routinely use raw water directly from rivers and dams.

4. Medical waste and pesticides are often dumped in unprepared locations, where leaching of pharmaceuticals and pesticides into groundwater sources is possible. The contamination of groundwater and retrieval and use of such water through boreholes, remain unexplored. We suggest a study on the presence of pharmaceuticals in borehole water due to leaching from medical waste dumping grounds. This may also be relevant to areas where illegal dumping of medical waste has been confirmed.

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# Appendix A

## WHO Guideline values for chemicals that are of health concern in drinking-water

Adapted from (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB; Who, 2011)

| Chemical | Guideline value | | Remarks |
|---|---|---|---|
| | mg/L | µg/L | |
| Acrylamide | 0.0005[a] | 0.5[a] | |
| Alachlor | 0.02[a] | 20[a] | |
| Aldicarb | 0.01 | 10 | Applies to aldicarb sulfoxide and aldicarb sulfone |
| Aldrin and dieldrin | 0.00003 | 0.03 | For combined aldrin plus dieldrin |
| Antimony | 0.02 | 20 | |
| Arsenic | 0.01 (A, T) | 10 (A, T) | |
| Atrazine and its chloro-s-triazine metabolites | 0.1 | 100 | |
| Barium | 0.7 | 700 | |
| Benzene | 0.01[a] | 10[a] | |
| Benzo[a]pyrene | 0.0007[a] | 0.7[a] | |
| Boron | 2.4 | 2400 | |
| Bromate | 0.01[a] (A, T) | 10[a] (A, T) | |
| Bromodichloromethane | 0.06[a] | 60a | |
| Bromoform | 0.1 | 100 | |
| Cadmium | 0.003 | 3 | |
| Carbofuran | 0.007 | 7 | |
| Carbon tetrachloride | 0.004 | 4 | |
| Chlorate | 0.7 (D) | 700 (D) | |
| Chlordane | 0.0002 | 0.2 | |
| Chlorine | 5 (C) | 5000 (C) | For effective disinfection, there should be a residual concentration of free chlorine of ≥0.5 mg/l after at least 30 min contact time at pH < 8.0. A chlorine residual should be maintained throughout the distribution system. At the point of delivery, the minimum residual concentration of free chlorine should be 0.2 mg/l. |

| | | | |
|---|---|---|---|
| Chlorite | 0.7 (D) | 700 (D) | |
| Chloroform | 0.3 | 300 | |
| Chlorotoluron | 0.03 | 30 | |
| Chlorpyrifos | 0.03 | 30 | |
| Chromium | 0.05 (P) | 50 (P) | For total chromium |
| Copper | 2 | 2000 | Staining of laundry and sanitary ware may occur below guideline value |
| Cyanazine | 0.0006 | 0.6 | |
| 2,4-Db | 0.03 | 30 | Applies to free acid |
| 2,4-DBc | 0.09 | 90 | |
| DDTd and metabolites | 0.001 | 1 | |
| Dibromoacetonitrile | 0.07 | 70 | |
| Dibromochloromethane | 0.1 | 100 | |
| 1,2-Dibromo-3-chloropropane | 0.001[a] | 1[a] | |
| 1,2-Dibromoethane | 0.00004[a] (P) | 0.4[a] (P) | |
| Dichloroacetate | 0.05[a] (D) | 50[a] (D) | |
| Dichloroacetonitrile | 0.02 (P) | 20 (P) | |
| 1,2-Dichlorobenzene | 1 (C) | 1 000 (C) | |
| 1,4-Dichlorobenzene | 0.3 (C) | 300 (C) | |
| 1,2-Dichloroethane | 0.03[a] | 30[a] | |
| 1,2-Dichloroethene | 0.05 | 50 | |
| Dichloromethane | 0.02 | 20 | |
| 1,2-Dichloropropane | 0.04 (P) | 40 (P) | |
| 1,3-Dichloropropene | 0.02[a] | 20[a] | |
| Dichlorprop | 0.1 | 100 | |
| Di(2-ethylhexyl)phthalate | 0.008 | 8 | |
| Dimethoate | 0.006 | 6 | |
| 1,4-Dioxane | 0.05[a] | 50[a] | Derived using tolerable daily intake approach as well as linearized multistage modelling |
| Edetic acid | 0.6 | 600 | Applies to the free acid |
| Endrin | 0.0006 | 0.6 | |
| Epichlorohydrin | 0.0004 (P) | 0.4 (P) | |
| Ethylbenzene | 0.3 (C) | 300 (C) | |
| Fenoprop | 0.009 | 9 | |

| | | | |
|---|---|---|---|
| Fluoride | 1.5 | 1500 | Volume of water consumed and intake from other sources should be considered when setting national standards |
| Hexachlorobutadiene | 0.0006 | 0.6 | |
| Hydroxyatrazine | 0.2 | 200 | Atrazine metabolite |
| Isoproturon | 0.009 | 9 | |
| Lead | 0.01 (A, T) | 10 (A, T) | |
| Lindane | 0.002 | 2 | |
| MCPAe | 0.002 | 2 | |
| | | | |
| Mecoprop | 0.01 | 10 | |
| Mercury | 0.006 | 6 | For inorganic mercury |
| Methoxychlor | 0.02 | 20 | |
| Metolachlor | 0.01 | 10 | |
| Microcystin-LR | 0.001 (P) | 1 (P) | For total microcystin-LR (free plus cell-bound) |
| Molinate | 0.006 | 6 | |
| Monochloramine | 3 | 3000 | |
| Monochloroacetate | 0.02 | 20 | |
| Nickel | 0.07 | 70 | |
| Nitrate (as NO3−) | 50 | 50000 | Short-term exposure |
| Nitrilotriacetic acid | 0.2 | 200 | |
| Nitrite (as NO2-) | 3 | 3000 | Short-term exposure |
| N-Nitrosodimethylamine | 0.0001 | 0.1 | |
| Pendimethalin | 0.02 | 20 | |
| Pentachlorophenol | 0.009[a] (P) | 9[a] (P) | |
| Selenium | 0.04 (P) | 40 (P) | |
| Simazine | 0.002 | 2 | |
| Sodium dichloroisocyanurate | 50 | 50000 | As sodium dichloroisocyanurate |
| | 40 | 40000 | As cyanuric acid |
| Styrene | 0.02 (C) | 20 (C) | |
| 2,4,5-Tf | 0.009 | 9 | |
| Terbuthylazine | 0.007 | 7 | |
| Tetrachloroethene | 0.04 | 40 | |
| Toluene | 0.7 (C) | 700 (C) | |

| | | | |
|---|---|---|---|
| Trichloroacetate | 0.2 | 200 | |
| Trichloroethene | 0.02 (P) | 20 (P) | |
| 2,4,6-Trichlorophenol | 0.2[a] (C) | 200[a] (C) | |
| Trifluralin | 0.02 | 20 | |
| Trihalomethanes | — | — | The sum of the ratio of the concentration of each to its respective guideline value should not exceed 1 |
| Uranium | 0.30 (P) | 30 (P) | Only chemical aspects of uranium addressed |
| Vinyl chloride | 0.0003[a] | 0.3[a] | |
| Xylenes | 0.5 (C) | 500 (C) | |

A provisional guideline value because calculated guideline value is below the achievable quantification level; **C**, concentrations of the substance at or below the health-based guideline value may affect the appearance, taste or odour of the water, leading to consumer complaints; **D**, provisional guideline value because disinfection is likely to result in the guideline value being exceeded; **P**, provisional guideline value because of uncertainties in the health database; **T**, provisional guideline value because calculated guideline value is below the level that can be achieved through practical treatment methods, source protection, etc.

**a** For substances that are considered to be carcinogenic, the guideline value is the concentration in drinking-water associated with an upper-bound excess lifetime cancer risk of 10−5 (one additional case of cancer per 100 000 of the population ingesting drinking-water containing the substance at the guideline value for 70 years). Concentrations associated with upper-bound estimated excess lifetime cancer risks of 10−4 and 10−6 can be calculated by multiplying and dividing, respectively, the guideline value by 10.

**b** 2,4-Dichlorophenoxyacetic acid.

**c** 2,4-Dichlorophenoxybutyric acid.

**d** Dichlorodiphenyltrichloroethane.

**e** 4-(2-Methyl-4-chlorophenoxy)acetic acid.

**f** 2,4,5-Trichlorophenoxyacetic acid

## Appendix B

## EPA Candidate Contaminant List 3

| Chemical Contaminants | | |
|---|---|---|
| **Substance Name** | **CASRN** | **Use** |
| 1,1,1,2-Tetrachloroethane | 630-20-6 | It is an industrial chemical used in the production of other substances. |
| 1,1-Dichloroethane | 75-34-3 | It is an industrial chemical used as a solvent. |
| 1,2,3-Trichloropropane | 96-18-4 | It is an industrial chemical used in paint manufacture. |
| 1,3-Butadiene | 106-99-0 | It is an industrial chemical used in rubber production. |
| 1,3-Dinitrobenzene | 99-65-0 | It is an industrial chemical and is used in the production of other substances. |
| 1,4-Dioxane | 123-91-1 | It is used as a solvent or solvent stabiliser in the manufacture and processing of paper, cotton, textile products, automotive coolant, cosmetics and shampoos. |
| 17alpha-estradiol | 57-91-0 | It is an estrogenic hormone and is used in pharmaceuticals. |
| 1-Butanol | 71-36-3 | It is used in the production of other substances, and as a paint solvent and food additive. |
| 2-Methoxyethanol | 109-86-4 | It is used in consumer products, such as synthetic cosmetics, perfumes, fragrances, hair preparations, and skin lotions. |
| 2-Propen-1-ol | 107-18-6 | It is used in the production of other substances, and in the manufacture of flavorings and perfumes. |
| 3-Hydroxycarbofuran | 16655-82-6 | It is a carbamate, and is a pesticide degradate. The parent, carbofuran, is used as an insecticide. |
| 4,4'-Methylenedianiline | 101-77-9 | It is used in the production of other substances, and as a corrosion inhibitor and curing agent for polyurethanes. |
| Acephate | 30560-19-1 | It is used as an insecticide. |
| Acetaldehyde | 75-07-0 | It is used in the production of other substances, and as a pesticide and food additive. |

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|---|---|---|
| Acetamide | 60-35-5 | It is used as a solvent, solubiliser, plasticiser, and stabiliser. |
| Acetochlor | 34256-82-1 | It is used as an herbicide for weed control on agricultural crops. |
| Acetochlor ethanesulfonic acid (ESA) | 187022-11-3 | Acetochlor ESA is an acetanilide pesticide degradate. The parent, acetochlor, is used as an herbicide for weed control on agricultural crops. |
| Acetochlor oxanilic acid (OA) | 184992-44-4 | Acetochlor OA is an acetanilide pesticide degradate. The parent, acetochlor, is used as an herbicide for weed control on agricultural crops. |
| Acrolein | 107-02-8 | It is used as an aquatic herbicide, rodenticide, and industrial chemical. |
| Alachlor ethanesulfonic acid (ESA) | 142363-53-9 | Alachlor ESA is an acetanilide pesticide degradate. The parent, alachlor, is used as an herbicide for weed control on agricultural crops. |
| Alachlor oxanilic acid (OA) | 171262-17-2 | Alachlor OA is an acetanilide pesticide degradate. The parent, alachlor, is used as an herbicide for weed control on agricultural crops. |
| alpha-Hexachlorocyclohexane | 319-84-6 | It is a component of benzene hexachloride (BHC) and was formerly used as an insecticide. |
| Aniline | 62-53-3 | It is used as an industrial chemical, as a solvent, in the synthesis of explosives, rubber products, and in isocyanates. |
| Bensulide | 741-58-2 | It is used as an herbicide. |
| Benzyl chloride | 100-44-7 | It is used in the production of other substances, such as plastics, dyes, lubricants, gasoline and pharmaceuticals. |
| Butylated hydroxyanisole | 25013-16-5 | It is used as a food additive (antioxidant). |
| Captan | 133-06-2 | It is used as a fungicide. |
| Chlorate | 14866-68-3 | Chlorate compounds are used in agriculture as defoliants or desiccants and may occur in drinking water related to use of disinfectants such as chlorine dioxide. |
| Chloromethane (Methyl chloride) | 74-87-3 | It is used as a foaming agent and in the production of other substances. |
| Clethodim | 110429-62-4 | It is used as an herbicide. |

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|---|---|---|
| Cobalt | 7440-48-4 | It is a naturally-occurring element and was formerly used as cobaltus chloride in medicines and as a germicide. |
| Cumene hydroperoxide | 80-15-9 | It is used as an industrial chemical and is used in the production of other substances. |
| Cyanotoxins (3)* | | Toxins naturally produced and released by cyanobacteria ("blue-green algae"). Various studies suggest three cyanotoxins for consideration: Anatoxin-a, Microcystin-LR, and Cylindrospermopsin. |
| Dicrotophos | 141-66-2 | It is used as an insecticide. |
| Dimethipin | 55290-64-7 | It is used as an herbicide and plant growth regulator. |
| Dimethoate | 60-51-5 | It is used as an insecticide on field crops, (such as cotton), orchard crops, vegetable crops, in forestry and for residential purposes. |
| Disulfoton | 298-04-4 | It is used as an insecticide. |
| Diuron | 330-54-1 | It is used as an herbicide. |
| equilenin | 517-09-9 | It is an estrogenic hormone and is used in pharmaceuticals. |
| equilin | 474-86-2 | It is an estrogenic hormone and is used in pharmaceuticals. |
| Erythromycin | 114-07-8 | It is used in pharmaceutical formulations as an antibiotic. |
| Estradiol (17-beta estradiol) | 50-28-2 | It is an estrogenic hormone and is used in pharmaceuticals. |
| estriol | 50-27-1 | It is an estrogenic hormone and is used in veterinary pharmaceuticals. |
| estrone | 53-16-7 | It is an estrogenic hormone and is used in veterinary and human pharmaceuticals. |
| Ethinyl Estradiol (17-alpha ethynyl estradiol) | 57-63-6 | It is an estrogenic hormone and is used in veterinary and human pharmaceuticals. |
| Ethoprop | 13194-48-4 | It is used as an insecticide. |
| Ethylene glycol | 107-21-1 | It is used as an antifreeze, in textile manufacture and is a cancelled pesticide. |
| Ethylene oxide | 75-21-8 | It is used as a fungicidal and insecticidal fumigant. |
| Ethylene thiourea | 96-45-7 | It is used in the production of other substances, such as for vulcanising polychloroprene (neoprene) and polyacrylate rubbers, and as a pesticide. |

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|---|---|---|
| Fenamiphos | 22224-92-6 | It is used as an insecticide. |
| Formaldehyde | 50-00-0 | It has been used as a fungicide, may be a disinfection by-product, and can occur naturally. |
| Germanium | 7440-56-4 | It is a naturally-occurring element and is commonly used as germanium dioxide in phosphors, transistors and diodes, and in electroplating. |
| Halon 1011 (bromochloromethane) | 74-97-5 | It is used as a fire-extinguishing fluid and to suppress explosions, as well as a solvent in the manufacturing of pesticides. May also occur as a disinfection by-product in drinking water. |
| HCFC-22 | 75-45-6 | It is used as a refrigerant, as a low-temperature solvent, and in fluorocarbon resins, especially in tetrafluoroethylene polymers. |
| Hexane | 110-54-3 | It is used as a solvent and is a naturally-occurring alkane. |
| Hydrazine | 302-01-2 | It is used in the production of other substances, such as rocket propellants, and as an oxygen and chlorine scavenging compound. |
| Mestranol | 72-33-3 | It is an estrogenic hormone and is used in veterinary and human pharmaceuticals. |
| Methamidophos | 10265-92-6 | It is used as an insecticide. |
| Methanol | 67-56-1 | It is used as an industrial solvent, a gasoline additive and also as anti-freeze. |
| Methyl bromide (Bromomethane) | 74-83-9 | It has been used as a fumigant as a fungicide. |
| Methyl tert-butyl ether | 1634-04-4 | It is used as an octane booster in gasoline, in the manufacture of isobutene and as an extraction solvent. |
| Metolachlor | 51218-45-2 | It is used as an herbicide for weed control on agricultural crops. |
| Metolachlor ethanesulfonic acid (ESA) | 171118-09-5 | Metolachlor ESA is an acetanilide pesticide degradate. The parent, metolachlor, is used as an herbicide for weed control on agricultural crops. |
| Metolachlor oxanilic acid (OA) | 152019-73-3 | Metolachlor OA is an acetanilide pesticide degradate. The parent, metolachlor, is used as an herbicide for weed control on agricultural crops. |
| Molinate | 2212-67-1 | It is used as an herbicide. |

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| Molybdenum | 7439-98-7 | It is a naturally-occurring element and is commonly used as molybdenum trioxide as a chemical reagent. |
| Nitrobenzene | 98-95-3 | It is used in the production of aniline, and also as a solvent in the manufacture of paints, shoe polishes, floor polishes, metal polishes, explosives, dyes, pesticides and drugs (such as acetaminophen), and in its re-distilled from (oil of mirbane) as an inexpensive perfume for soaps. |
| Nitroglycerin | 55-63-0 | It is used in pharmaceuticals, in the production of explosives, and in rocket propellants. |
| N-Methyl-2-pyrrolidone | 872-50-4 | It is a solvent in the chemical industry, and is used for pesticide application and in food packaging materials. |
| N-nitrosodiethylamine (NDEA) | 55-18-5 | It is a nitrosamine used as an additive in gasoline and in lubricants, as an antioxidant, as a stabilizer in plastics, and also may be a disinfection byproduct. |
| N-nitrosodimethylamine (NDMA) | 62-75-9 | It is a nitrosamine and has been formerly used in the production of rocket fuels, is used as an industrial solvent and an anti-oxidant, and also may be a disinfection byproduct. |
| N-nitroso-di-n-propylamine (NDPA) | 621-64-7 | It is a nitrosamine and may be a disinfection byproduct. |
| N-Nitrosodiphenylamine | 86-30-6 | It is a nitrosamine chemical reagent that is used as a rubber and polymer additive and may be a disinfection byproduct. |
| N-nitrosopyrrolidine (NPYR) | 930-55-2 | It is a nitrosamine used as a research chemical and may be a disinfection byproduct. |
| Norethindrone (19-Norethisterone) | 68-22-4 | It is a progresteronic hormone used in pharmaceuticals. |
| n-Propylbenzene | 103-65-1 | It is used in the manufacture of methylstyrene, in textile dyeing, and as a printing solvent, and is a constituent of asphalt and naptha. |
| o-Toluidine | 95-53-4 | It is used in the production of other substances, such as dyes, rubber, pharmaceuticals and pesticides. |
| Oxirane, methyl- | 75-56-9 | It is an industrial chemical used in the production of other substances. |
| Oxydemeton-methyl | 301-12-2 | It is used as an insecticide. |
| Oxyfluorfen | 42874-03-3 | It is used as an herbicide. |

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|---|---|---|
| Perchlorate | 14797-73-0 | It is both a naturally occurring and human-made chemical. Perchlorate is used to manufacture fireworks, explosives, flares and rocket propellant. |
| Perfluorooctane sulfonic acid (PFOS) | 1763-23-1 | PFOS was used in fire fighting foams and various surfactant uses; few of which are still ongoing because no alternatives are available. |
| Perfluorooctanoic acid (PFOA) | 335-67-1 | PFOA is used in the manufacture of fluoropolymers, substances which provide non-stick surfaces on cookware and waterproof, breathable membranes for clothing |
| Permethrin | 52645-53-1 | It is used as an insecticide. |
| Profenofos | 41198-08-7 | It is used as an insecticide and an acaricide. |
| Quinoline | 91-22-5 | It is used in the production of other substances, and as a pharmaceutical (anti-malarial) and as a flavoring agent. |
| RDX (Hexahydro-1,3,5-trinitro-1,3,5-triazine) | 121-82-4 | It is used as an explosive. |
| sec-Butylbenzene | 135-98-8 | It is used as a solvent for coating compositions, in organic synthesis, as a plasticiser and in surfactants. |
| Strontium | 7440-24-6 | It is naturally-occurring element and is used as strontium carbonate in pyrotechnics, in steel production, as a catalyst and as a lead scavenger. |
| Tebuconazole | 107534-96-3 | It is used as a fungicide. |
| Tebufenozide | 112410-23-8 | It is used as an insecticide. |
| Tellurium | 13494-80-9 | It is a naturally-occurring element and is commonly used as sodium tellurite in bacteriology and medicine. |
| Terbufos | 13071-79-9 | It is used as an insecticide. |
| Terbufos sulfone | 56070-16-7 | Terbufos sulfone is a phosphorodithioate pesticide degradate. The parent, terbufos, is used as an insecticide. |
| Thiodicarb | 59669-26-0 | It is used as an insecticide. |
| Thiophanate-methyl | 23564-05-8 | It is used as a fungicide. |

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| Toluene diisocyanate | 26471-62-5 | It is used in the manufacture of plastics. |
| Tribufos | 78-48-8 | It is used as an insecticide and as a cotton defoliant. |
| Triethylamine | 121-44-8 | It is used in the production of other substances, and as a stabilizer in herbicides and pesticides, in consumer products, in food additives, in photographic chemicals and in carpet cleaners. |
| Triphenyltin hydroxide (TPTH) | 76-87-9 | It is used as a pesticide. |
| Urethane | 51-79-6 | It is used as a paint ingredient. |
| Vanadium | 7440-62-2 | It is a naturally-occurring element and is commonly used as vanadium pentoxide in the production of other substances and as a catalyst. |
| Vinclozolin | 50471-44-8 | It is used as a fungicide. |
| Ziram | 137-30-4 | It is used as a fungicide. |

# Appendix C
# USGS Emerging Contaminant List

| COMPOUND | CASRN | REPORTING LEVEL | MATRIX | USE |
|---|---|---|---|---|
| 1,4-dichlorobenzene | 106-46-7 | 0.5 ug/L | water | deodoriser |
| 1-methylnaphthalene | 90-12-0 | 0.5 ug/L | water | PAH |
| 2,6-dimethylnaphthalene | 581-42-0 | 0.5 ug/L | water | PAH |
| 2-methylnaphthalene | 91-57-6 | 0.5 ug/L | water | PAH |
| 3-beta-coprostanol | 360-68-9 | 2.0 ug/L | water | fecal steroid |
| 3-methyl-1(H)-indole (Skatole) | 83-34-1 | 1.0 ug/L | water | fragrance |
| 3-tert-butyl-4-hydroxy anisole (BHA) | 25013-16-5 | 5.0 ug/L | water | antioxidant |
| 4-cumylphenol | 599-64-4 | 1.0 ug/L | water | nonionic detergent metabolite |
| 4-n-octylphenol | 1806-26-4 | 1.0 ug/L | water | nonionic detergent metabolite |
| 4-tert-octylphenol | 140-66-9 | 1.0 ug/L | water | nonionic detergent metabolite |
| 5-methyl-1H-benzotriazole | 136-85-6 | 2.0 ug/L | water | antiocorrosive |
| acetophenone | 98-86-2 | 0.5 ug/L | water | fragrance |
| acetyl hexamethyl tetrahydronaphthalene (AHTN) | 21145-77-7 | 0.5 ug/L | water | fragrance |
| anthracene | 120-12-7 | 0.5 ug/L | water | PAH |
| anthraquinone | 84-65-1 | 0.5 ug/L | water | pesticide |
| benzo[a]pyrene | 50-32-8 | 0.5 ug/L | water | PAH |
| benzophenone | 119-61-9 | 0.5 ug/L | water | plasticiser |
| beta-sitosterol | 83-46-5 | 2.0 ug/L | water | plant steroid |
| beta-stigmastanol | 19466-47-8 | 2.0 ug/L | water | plant steroid |
| bisphenol A | 80-05-7 | 1.0 ug/L | water | plasticiser |
| bromacil | 314-40-9 | 0.5 ug/L | water | herbicide |

| | | | | |
|---|---|---|---|---|
| bromoform | 75-25-2 | 0.5 ug/L | water | disinfectant |
| caffeine | 58-08-2 | 0.5 ug/L | water | stimulant |
| caffeine-C13 | - | pct | water | *surrogate standard* |
| camphor | 76-22-2 | 0.5 ug/L | water | flavorant |
| carbaryl | 63-25-2 | 1.0 ug/L | water | insecticide |
| carbazole | 86-74-8 | 0.5 ug/L | water | PAH |
| chlorpyrifos | 2921-88-2 | 0.5 ug/L | water | insecticide |
| cholesterol | 57-88-5 | 2.0 ug/L | water | plant/animal steroid |
| cotinine | 486-56-6 | 1.0 ug/L | water | nicotine metabolite |
| decafluorobiphenyl | - | pct | water | polymer |
| diazinon | 333-41-5 | 0.5 ug/L | water | insecticide |
| dichlorvos | 62-73-7 | 1.0 ug/L | water | insecticide |
| d-limonene | 5989-27-5 | 0.5 ug/L | water | fungicide |
| fluoranthene, d10 | - | pct | water | *surrogate standard* |
| fluoranthene | 206-44-0 | 0.5 ug/L | water | PAH |
| hexadydrohexamethylcyclopentabenzopyran (HHCB) | 1222-05-5 | 0.5 ug/L | water | fragrance |
| indole | 120-72-9 | 0.5 ug/L | water | pesticide inert ingredient |
| isoborneol | 124-76-5 | 0.5 ug/L | water | fragrance |
| isophorone | 78-59-1 | 0.5 ug/L | water | solvent |
| isopropylbenzene (cumene) | 98-82-8 | 0.5 ug/L | water | solvent |
| isoquinoline | 119-65-3 | 0.5 ug/L | water | fragrance |
| menthol | 89-78-1 | 0.5 ug/L | water | flavorant |
| metalaxyl | 57837-19-1 | 0.5 ug/L | water | pesticide |
| methyl salicylate | 119-36-8 | 0.5 ug/L | water | liniment |
| metolachlor | 51218-45-2 | 0.5 ug/L | water | herbicide |
| N,N-diethyl-meta-toluamide (DEET) | 134-62-3 | 0.5 ug/L | water | insect repellant |
| naphthalene | 91-20-3 | 0.5 ug/L | water | PAH |
| nonylphenol, diethoxy-(total) (NPEO2) | 26027-38-2 | 5.0 ug/L | water | nonionic detergent metabolite |

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|---|---|---|---|---|
| octylphenol, diethoxy- (OPEO2) | 26636-32-8 | 1.0 ug/L | water | nonionic detergent metabolite |
| octylphenol, monoethoxy- (OPEO1) | 26636-32-8 | 1.0 ug/L | water | nonionic detergent metabolite |
| para-nonylphenol (total) | 84852-15-3 | 5.0 ug/L | water | nonionic detergent metabolite |
| p-cresol | 106-44-5 | 1.0 ug/L | water | antioxidant |
| pentachlorophenol | 87-86-5 | 2.0 ug/L | water | pesticide |
| phenanthrene | 85-01-8 | 0.5 ug/L | water | PAH |
| phenol | 108-95-2 | 0.5 ug/L | water | disinfectant |
| prometon | 1610-18-0 | 0.5 ug/L | water | herbicide |
| pyrene | 129-00-0 | 0.5 ug/L | water | PAH |
| tetrachloroethylene | 127-18-4 | 0.5 ug/L | water | solvent, degreaser |
| tri(2-butoxyethyl)phosphate | 78-51-3 | 0.5 ug/L | water | fire retardant |
| tri(2-chloroethyl)phosphate | 115-96-8 | 0.5 ug/L | water | fire retardant |
| tri(dichlorisopropyl)phosphate | 13674-87-8 | 0.5 ug/L | water | fire retardant |
| tributyl phosphate | 126-73-8 | 0.5 ug/L | water | fire retardant |
| triclosan | 3380-34-5 | 1.0 ug/L | water | antimicrobial disinfectant |
| triethyl citrate (ethyl citrate) | 77-93-0 | 0.5 ug/L | water | plasticiser |
| triphenyl phosphate | 115-86-6 | 0.5 ug/L | water | plasticiser |
| | | | | |
| 1,4-dichlorobenzene | 106-46-7 | 50 ug/kg | sediment | deodoriser |
| 1-methylnaphthalene | 90-12-0 | 50 ug/kg | sediment | PAH |
| 2,6-dimethylnaphthalene | 581-42-0 | 50 ug/kg | sediment | PAH |
| 2-methylnaphthalene | 91-57-6 | 50 ug/kg | sediment | PAH |
| 3,4-dichlorophenyl isocyanate | 102-36-3 | 100 ug/kg | sediment | plastic additive |
| 3-beta-coprostanol | 360-68-9 | 500 ug/kg | sediment | fecal steroid |
| 3-methyl-1(H)-indole (Skatole) | 83-34-1 | 50 ug/kg | sediment | fragrance |
| 3-tert-butyl-4-hydroxy anisole (BHA) | 25013-16-5 | 100 ug/kg | sediment | antioxidant |

| | | | | |
|---|---|---|---|---|
| 4-cumylphenol | 599-64-4 | 50 ug/kg | sediment | nonionic detergent metabolite |
| 4-n-octylphenol | 1806-26-4 | 50 ug/kg | sediment | nonionic detergent metabolite |
| 4-tert-octylphenol | 140-66-9 | 50 ug/kg | sediment | nonionic detergent metabolite |
| acetophenone | 98-86-2 | 100 ug/kg | sediment | fragrance |
| acetyl hexamethyl tetrahydro-naphthalene (AHTN) | 21145-77-7 | 50 ug/kg | sediment | fragrance |
| anthracene | 120-12-7 | 50 ug/kg | sediment | PAH |
| anthraquinone | 84-65-1 | 50 ug/kg | sediment | pesticide |
| atrazine | 1912-24-9 | 100 ug/kg | sediment | herbicide |
| benzo[a]pyrene | 50-32-8 | 50 ug/kg | sediment | PAH |
| benzophenone | 119-61-9 | 50 ug/kg | sediment | plasticiser |
| beta-sitosterol | 83-46-5 | 500 ug/kg | sediment | plant steroid |
| beta-stigmastanol | 19466-47-8 | 500 ug/kg | sediment | plant steroid |
| bisphenol A | 80-05-7 | 50 ug/kg | sediment | plasticiser |
| bromacil | 314-40-9 | 500 ug/kg | sediment | herbicide |
| camphor | 76-22-2 | 50 ug/kg | sediment | flavorant |
| carbazole | 86-74-8 | 50 ug/kg | sediment | PAH |
| chlorpyrifos | 2921-88-2 | 50 ug/kg | sediment | insecticide |
| cholesterol | 57-88-5 | 250 ug/kg | sediment | plant/animal steroid |
| diazinon | 333-41-5 | 50 ug/kg | sediment | insecticide |
| diethyl phthalate | 84-66-2 | 100 ug/kg | sediment | plastic additive |
| diethylhexyl phthalate | 117-81-7 | 250 ug/kg | sediment | plastic additive |
| d-limonene | 5989-27-5 | 50 ug/kg | sediment | fungicide |
| fluoranthene | 206-44-0 | 50 ug/kg | sediment | PAH |
| hexahydrohexamethylcyclopentabenzopyran (HHCB) | 1222-05-5 | 50 ug/kg | sediment | fragrance |

| | | | | |
|---|---|---|---|---|
| indole | 120-72-9 | 50 ug/kg | sediment | pesticide inert ingredient |
| isoborneol | 124-76-5 | 50 ug/kg | sediment | fragrance |
| isophorone | 78-59-1 | 50 ug/kg | sediment | solvent |
| isopropylbenzene (cumene) | 98-82-8 | 100 ug/kg | sediment | solvent |
| isoquinoline | 119-65-3 | 100 ug/kg | sediment | fragrance |
| menthol | 89-78-1 | 50 ug/kg | sediment | flavorant |
| metalaxyl | 57837-19-1 | 50 ug/kg | sediment | pesticide |
| methyl salicylate | 119-36-8 | 50 ug/kg | sediment | liniment |
| metolachlor | 51218-45-2 | 50 ug/kg | sediment | herbicide |
| N,N-diethyl-meta-toluamide (DEET) | 134-62-3 | 50 ug/kg | sediment | insect repellant |
| naphthalene | 91-20-3 | 50 ug/kg | sediment | PAH |
| nonylphenol, diethoxy-(total) (NPEO2) | 26027-38-2 | 1000 ug/kg | sediment | nonionic detergent metabolite |
| nonylphenol, monoethoxy-(total) (NPEO1) | | 500 ug/kg | sediment | nonionic detergent metabolite |
| octylphenol, diethoxy- (OPEO2) | 26636-32-8 | 50 ug/kg | sediment | nonionic detergent metabolite |
| octylphenol, monoethoxy- (OPEO1) | 26636-32-8 | 250 ug/kg | sediment | nonionic detergent metabolite |
| para-nonylphenol (total) | 84852-15-3 | 250 ug/kg | sediment | nonionic detergent metabolite |
| para-cresol | 106-44-5 | 500 ug/kg | sediment | antioxidant |
| pentachlorophenol | 87-86-5 | 500 ug/kg | sediment | pesticide |
| phenanthrene | 85-01-8 | 50 ug/kg | sediment | PAH |
| phenol | 108-95-2 | 50 ug/kg | sediment | disinfectant |
| prometon | 1610-18-0 | 50 ug/kg | sediment | herbicide |
| pyrene | 129-00-0 | 50 ug/kg | sediment | PAH |
| 2,2',4,4'-tetrabromodiphenyl ether | 40088-47-9 | 50 ug/kg | sediment | fire retardant |
| tri(2-butoxyethyl)phosphate | 78-51-3 | 100 ug/kg | sediment | fire retardant |
| tri(2-chloroethyl)phosphate | 115-96-8 | 100 ug/kg | sediment | fire retardant |
| tri(dichlorisopropyl)phosphate | 13674-87-8 | 100 ug/kg | sediment | fire retardant |

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|---|---|---|---|---|
| tributyl phosphate | 126-73-8 | 50 ug/kg | sediment | fire retardant |
| triclosan | 3380-34-5 | 50 ug/kg | sediment | antimicrobial disinfectant |
| triphenyl phosphate | 115-86-6 | 50 ug/kg | sediment | plasticiser |
| | | | | |
| 1,7-dimethylxanthine | 611-59-6 | 0.144 ug/L | water | caffeine metabolite |
| codeine | 76-57-3 | 0.015 ug/L | water | analgesic |
| caffeine | 58-08-2 | 0.016 ug/L | water | stimulant |
| thiabendazole | | 0.011 ug/L | water | fungicide |
| albuterol (Salbutamol) | 18559-94-9 | 0.023 ug/L | water | antiasthmatic |
| acetaminophen | 103-90-2 | 0.036 ug/L | water | antipyretic |
| cotinine | 486-56-6 | 0.014 ug/L | water | nicotine metabolite |
| dehydronifedipine | 67035-22-7 | 0.015 ug/L | water | nifedipine metabolite |
| carbamazapine | | 0.011 ug/L | water | anticonvulsant |
| trimethoprim | 738-70-5 | 0.013 ug/L | water | antibiotic |
| warfarin | 81-81-2 | 0.012 ug/L | water | anticoagulant |
| diphenhydramine | | 0.015 ug/L | water | antihistamine |
| sulfamethoxazole | 723-46-6 | 0.064 ug/L | water | antibiotic |
| diltiazem | 42399-41-7 | 0.016 ug/L | water | antihypertensive |
| ibuprofen | 15687-27-1 | 0.042 ug/L | water | antiinflammatory |
| ranitidine | 66357-35-5 | 0.013 ug/L | water | antacid |
| cimetidine | 51481-61-9 | 0.012 ug/L | water | antacid |
| fluoxetine | 54910-89-3 | 0.014 ug/L | water | antidepressant |
| gemfibrozil | 25812-30-0 | 0.013 ug/L | water | antihyperlipidemic |
| naproxen | | not detectable | water | antiinflammatory |
| erythromycin | 114-07-8 | 0.009 ug/L | water | antibiotic |
| azithromycin | | 0.004 ug/L | water | antibiotic |
| miconazole | | 0.018 ug/L | water | antifungal |
| metformin | 657-24-9 | N/D | water | antidiabetic |
| | | | | |
| 1,7-dimethylxanthine | 611-59-6 | | sediment | caffeine metabolite |

| | | | | |
|---|---|---|---|---|
| codeine | 76-57-3 | | sediment | analgesic |
| caffeine | 58-08-2 | | sediment | stimulant |
| thiabendazole | | | sediment | fungicide |
| albuterol (Salbutamol) | 18559-94-9 | | sediment | antiasthmatic |
| acetaminophen | 103-90-2 | | sediment | antipyretic |
| cotinine | 486-56-6 | | sediment | nicotine metabolite |
| dehydronifedipine | 67035-22-7 | | sediment | nifedipine metabolite |
| carbamazapine | | | sediment | anticonvulsant |
| trimethoprim | 738-70-5 | | sediment | antibiotic |
| warfarin | 81-81-2 | | sediment | anticoagulant |
| diphenhydramine | | | sediment | antihistamine |
| sulfamethoxazole | 723-46-6 | | sediment | antibiotic |
| diltiazem | 42399-41-7 | | sediment | antihypertensive |
| ibuprofen | 15687-27-1 | | sediment | antiinflammatory |
| ranitidine | 66357-35-5 | | sediment | antacid |
| cimetidine | 51481-61-9 | | sediment | antacid |
| fluoxetine | 54910-89-3 | | sediment | antidepressant |
| gemfibrozil | 25812-30-0 | | sediment | antihyperlipidemic |
| erythromycin | 114-07-8 | | sediment | antibiotic |
| miconazole | | | sediment | antifungal |
| metformin | 657-24-9 | | sediment | antidiabetic |
| | | | | |
| ampicillin | 69-83-4 | 0.01 ug/L | water | antibiotic |
| cefotaxime | 63527-52-6 | 0.01 ug/L | water | antibiotic |
| cloxacillin | 61-72-3 | 0.01 ug/L | water | antibiotic |
| oxacillin | 66-79-5 | 0.01 ug/L | water | antibiotic |
| penicillin G | 61-33-6 | 0.01 ug/L | water | antibiotic |
| penicillin V | 87-08-1 | 0.01 ug/L | water | antibiotic |
| | | | | |
| erythromycin | 114-07-8 | 0.01 ug/L | water | antibiotic |
| anhydro-erythromycin | na | 0.01 ug/L | water | erythromycin degradate |

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|---|---|---|---|---|
| lincomycin | 154-21-2 | 0.01 ug/L | water | antibiotic |
| ormetoprim | 6981-18-6 | 0.01 ug/L | water | antibiotic |
| roxithromycin | 80214-83-1 | 0.01 ug/L | water | antibiotic |
| trimethoprim | 738-70-5 | 0.01 ug/L | water | antibiotic |
| tylosin | 1401-69-0 | 0.01 ug/L | water | antibiotic |
| virginiamycin M | 21411-53-0 | 0.01 ug/L | water | antibiotic |
| | | | | |
| carbadox | 6804-07-5 | 0.005 ug/L | water | antibiotic |
| ciprofloxacin | 85721-33-1 | 0.005 ug/L | water | antibiotic |
| clinafloxacin | 105956-97-6 | 0.005 ug/L | water | antibiotic |
| flumequine | 42835-25-6 | 0.005 ug/L | water | antibiotic |
| lomefloxacin | 98079-51-7 | 0.005 ug/L | water | antibiotic |
| norfloxacin | 70458-96-7 | 0.005 ug/L | water | antibiotic |
| ofloxacin | 82419-36-1 | 0.005 ug/L | water | antibiotic |
| oxolinic acid | 14698-29-4 | 0.005 ug/L | water | antibiotic |
| sarafloxacin | 98105-99-8 | 0.005 ug/L | water | antibiotic |
| | | | | |
| sulfachloropyridazine | 80-32-0 | 0.005 ug/L | water | antibiotic |
| sulfadiazine | 68-35-9 | 0.005 ug/L | water | antibiotic |
| sulfadimethoxine | 122-11-2 | 0.005 ug/L | water | antibiotic |
| sulfamerazine | 127-79-7 | 0.005 ug/L | water | antibiotic |
| sulfamethazine | 57-68-1 | 0.005 ug/L | water | antibiotic |
| sulfamethoxazole | 723-46-4 | 0.005 ug/L | water | antibiotic |
| sulfathiazole | 72-14-0 | 0.005 ug/L | water | antibiotic |
| | | | | |
| chlorotetracycline | 57-62-5 | 0.01 ug/L | water | antibiotic |
| anhydro-chlorotetracycline | 4497-08-9 | 0.01 ug/L | water | chlorotetracycline degradate |
| epi-anhydro-chlorotetracycline | 4497-08-9 | 0.01 ug/L | water | chlorotetracycline degradate |
| epi-chlorotetracycline | 14297-93-9 | 0.01 ug/L | water | chlorotetracycline degradate |

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|---|---|---|---|---|
| iso-chlorotetracycline | 514-53-4 | 0.01 ug/L | water | chlorotetracycline degradate |
| demeclocycline | 64-73-3 | 0.01 ug/L | water | antibiotic |
| doxycycline | 564-25-0 | 0.01 ug/L | water | antibiotic |
| minocycline | 10118-90-8 | 0.01 ug/L | water | antibiotic |
| oxytetracycline | 79-57-2 | 0.01 ug/L | water | antibiotic |
| epi-oxytetracycline | 35259-39-3 | 0.01 ug/L | water | oxytetracycline degradate |
| tetracycline | 60-54-8 | 0.01 ug/L | water | antibiotic |
| anhydro-tetracycline | 13803-65-1 | 0.01 ug/L | water | tetracycline degradate |
| epi-anhydro-tetracycline | 4465-65-0 | 0.01 ug/L | water | tetracycline degradate |
| epi-tetracycline | 23313-80-6 | 0.01 ug/L | water | tetracycline degradate |
| | | | | |
| cis-androsterone | 53-41-8 | | water | urinary steroid |
| bisphenol A | 80-05-7 | | water | plasticiser |
| cholesterol | 57-88-5 | | water | plant/animal steroid |
| 3-beta-coprostanol | 360-68-9 | | water | animal fecal steroid |
| equilenin | 517-09-9 | | water | hormone replacement |
| equilin | 474-86-2 | | water | hormone replacement |
| 17-alpha-estradiol | 57-91-0 | | water | reproductive hormone |
| 17-beta-estradiol | 50-28-2 | | water | reproductive hormone |
| estriol | 50-27-1 | | water | reproductive hormone |
| estrone | 53-16-7 | | water | reproductive hormone |
| ethylenediaminetetraacetic acid | 60-00-4 | | water | complexing agent |
| 4-ethylphenol | 123-07-9 | | water | plasticiser |
| 17-alpha-ethynylestradiol | 57-63-6 | | water | ovulation inhibitor |
| mestranol | 72-33-3 | | water | ovulation inhibitor |
| nitrilotriacetic acid | 139-13-9 | | water | complexing agent |
| 4-nonylphenol | 25154-52-3 | | water | surfactant metabolite |
| 4-nonylphenolmonoethoxylate | 9016-45-9 | | water | surfactant metabolite |
| 4-nonylphenoldiethoxylate | | | water | surfactant metabolite |
| 4-nonylphenoltriethoxylate | | | water | surfactant metabolite |

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|---|---|---|---|---|
| 4-nonylphenoltetraethoxylate | | | water | surfactant metabolite |
| 4-nonylphenolmonoethoxycarboxylate | 3115-49-9 | | water | surfactant metabolite |
| 4-nonylphenoldiethoxycarboxylate | 106807-78-7 | | water | surfactant metabolite |
| 4-nonylphenoltriethoxycarboxylate | | | water | surfactant metabolite |
| 4-nonylphenoltetraethoxycarboxylate | | | water | surfactant metabolite |
| 19-norethisterone | 68-22-4 | | water | ovulation inhibitor |
| 4-normal-octylphenol | 1806-26-4 | | water | plasticiser |
| 4-tert-octylphenol | 140-66-9 | | water | surfactant metabolite |
| 4-tert-octylphenolmonoethoxylate | 9036-19-5 | | water | surfactant metabolite |
| 4-tert-octylphenoldiethoxylate | | | water | surfactant metabolite |
| 4-tert-octylphenoltriethoxylate | | | water | surfactant metabolite |
| 4-tert-octylphenoltetraethoxylate | | | water | surfactant metabolite |
| 4-tert-pentylphenol | 80-46-6 | | water | plasticiser |
| progesterone | 57-83-0 | | water | reproductive hormone |
| 4-propylphenol | 645-56-7 | | water | plasticiser |
| testosterone | 58-22-0 | | water | reproductive hormone |
| triclosan | 3380-34-5 | | water | antimicrobial disinfectant |

# Appendix D
# Compounds Included in Qualitative Screen of Tap Water

α-Hydroxyalprazolam
α-Hydroxytriazolam
17-α-ethynylestradiol
2-(2-Methyl-4-chlorophenoxy)propanoic acid
2,4-Dichlorophenoxyacetic acid
2,4-Dichlorophenoxyacetic acid
2-Amino-5-nitrobenzophenone
2-Hydroxyethylflurazepam
2-Methyl-4-chlorophenoxyacetic acid
3,4-Methylenedioxyamphetamine
3,4-Methylenedioxy-methamphetamine
3,5-Xylyl methylcarbamate
3-Acetyldeoxynivalenol
3-Hydroxycarbofuran
4-(2,4-Dichlorophenoxy) butyric acid
4-(4-Chloro-o-tolyloxy)butyric acid
5-(p-Methylphenyl)-phenylhydantoin
6-Mercaptourine
6-O-Monoacetylmorphine
7-Aminoclonazepam
7-Aminoflunitrazepam
7-Aminonitrazepam
9-Hydroxyrisperidone
Abamectin
Aceclidine
Acephate
Acepromazine
Acetamiprid
Acetazolamide
Acetylsalicylic Acid
Aciclovir
Acrylamide
Adenine
Adenosine
Adrenalone
Afla B1
Afla B2
Afla G1
Afla G2
Ajmaline
Alachlor
Alanycarb
Aldicarb
Aldicarb Sulfoxide
Aldicarb Sulphone
Alprazolam
Alprenolol
Alprostadil
Amantadine
Ametryn
Amiloride
Aminocarb
Aminophenazone
Aminopromazine
Aminosalicylic acid 5-
Amiodarone
Amiphenazole
Amitriptylin
Amobarbital
Amoxicillin
Amoxicillin
Amphetamine
Apomorphine
Aprinidine
Atenolol
Atorvastatin
Atorvastatin
Atrazine
Atropine
Azelaic Acid
Azinphos methyl
Azinphos-ethyl
Azoxystrobin
Aztreonam
Aztreonam
Befunolol
Benalaxyl
Bendiacarb
Benfuracarb
Benomyl
Benserazid
Bentazon
Benzamidosalicyclic acid 4-
Benzatropine
Benzocaine
Benzoctamine
Benzoylecgonine
Benzthiazide
Berberine
Betaxolol
Bezafibrate
Bezafibrate
Biperiden
Bisoprolol
Bitertanol
Boscalid
Brallobarbital
Bromacil
Bromazepam
Bromocriptine
Bromoxynil
Brompheniramine
Bromuconazole
Bucetin
Bunitrolol
Bupirimate
Bupivacaine
Bupranolol
Buprenorphine
Buprofezine
Buspirone
Butaperazine
Butocarboxim
Butocarboxim-sulfoxide
Butoxycarboxim
Cadusafos
Caffeine
Carazolol
Carbamazepine
Carbaryl
Carbendazim
Carbenoxolone
Carbetamide
Carbinoxamine
Carbofuran
Carbosulfan
Carboxin
Carbuterol
Carteolol
Carvedilol
Celiprolol
Cetirizine
Chlorcyclizine
Chlordiazepoxide
Chlorfenvinphos
Chloridazon
Chloropropham
Chlorothiazide
Chlorotoluron
Chlorphenethiazine
Chlorpheniramine
Chlorpromazine
Chlorprothixene
Chlorpyrifos
Chlorsulfuron
Chlortoluron
Cilazapril
Cimetidine
Cinchonine
Cinnarizine
Citalopram
Clemastine
Clenbuterol
Clobazam
Clobutinol
Clofentezine
Clomazone
Clomethiazole
Clomipramine
Clonazepam

Clonidine
Clopyralid
Clothianidin
Clozapine
Cocaine
Codeine
Cortisone
Coumaphos
Coumatetralyl
Cyanazine
Cyanuric acid
Cyclicine
Cymoxanil
Cyproconazole
Cyprodinil
Demeton-S-methyl
Demeton-S-methyl-sulfone
Desalkylflurazepam
Desethyl atrazine
Desipramine
Desisopropyl atrazine
Desmedipham
Desmethylclomipramine
Desmethylflunitrazepam
Dextromethorphan
Dextropropoxyphene
Diazepam
Diazinon
Dicamba
Dichlorvos
Dicrotophos
Difenoconazole
Diflubenzuron
Diflubenzuron
Diflufenican
Dihydrocodeine
Dihydroergotamine
Dilazep
Diltiazem
Dimefuron
Dimepiperate
Dimethachlor
Dimethenamide
Dimethoate
Dimethomorph
Dinoprost
Dinoterb
Dioxacarb
Diphenhydramine
Diphenylamine
Dipyridamole
Disopyramide
Disulfoton
Diuron
Dixyrazine
Dodine
Doxapram
Doxepin
Ecgoninemethylester
Embutramide
Enalapril
Ephedrine
Epinephrine
Epoxiconazole
Eprosartan
Esmolol
Esprocarb
Estazolam
Estradiol
Estrone
Ethenzamide
Ethiofencarb
Ethiofencarb-sulfone
Ethiofencarb-sulfoxide
Ethyl dipropyl(thiocarbamate)
Ethyl glucuronide
Ethyl sulfate
Etofenprox
Etoxazole
Famoxadone
Famphur
Felodipine
Fenarimol
Fenazaquin
Fenbuconazole
Fendiline
Fenetylline
Fenfluramine
Fenhexamid
Fenobucarb
Fenoprop
Fenothiocarb
Fenoxap-p-ethyl
Fenoxycarb
Fenpropimorph
Fenpyroximate
Fentanyl
Fenthion
Fenuron
Fexofenadine
Flazasulfuron
Flecainide
Florosulam
Fluazifop
Fluazifop-p-butyl
Fluazinam
Fluconazole
Fludioxonil
Flufenacet
Flufenoxuron
Flumeturon
Flunitrazepam
Fluometuron
Fluoxetine
Fluoxypyr
Fluphenazine
Flurazepam
Fluroxypyr
Flusilazole
Fluvoxamine
Fosthiazate
Furathiocarb
Furosemid
Gabapentin
Gallopamil
Glibenclamide
Glibornuride
Glimepiride
Glipizide
Gliquidone
Haloperidol
Haloxyfop
Haloxyfop methyl
Heptenophos
Heroin
Hexaconazole
Hexaflumuron
Hexazinone
Hexythiazox
Hydrochlorothiazide
Hydrocodone
Hydrocortisone
Hydromorphone
Hydroxyzine
Imazalil
Imazamox
Imazapyr
Imazapyr
Imidacloprid
Imipramine
Indomethacin
Indoprofen
Indoxacarb
Ioxynil
Iprodione
Iprovalicarb
Irbesartan
Isofenfos
Isoprocarb
Isoproturon
Isoxaflutole
Ketamine
Ketoprofen
Ketorolac
Lamotrigine
Lenacil
Lercanidipine
Levocabastine
Levomepromazine
Lidocaine
Linuron
Lisinopril
Loperamide
Loratadine
Lorazepam
Lormetazepam
Losartan
Lufenuron
Lysergide
Malaoxon
Malathion
Maprotiline
Mecarbam

Meclizine
Medazepam
Meloxicam
Melperone
Mepanipyrim
Mepindolol
Mescaline
Mesoridazine
Metaclazepam
Metalaxyl
Metamfepramone
Metamitron
Metamphetamine
Metazachlor
Metformin
Methabenzthiazuron
Methadone
Methamidophos
Methiocarb
Methiocarb-sulfone
Methomyl
Methoxyfenozide
Methylphenidate
Metipranolol
Metobromuron
Metolachlor
Metoprolol
Metosulam
Metoxuron
Metribuzin
Metronidazole
Mevinphos
Mexiletine
Mianserin
Miconazole
Microcystin LA
Microcystin LF
Microcystin LR
Microcystin LW
Microcystin LY
Microcystin RR
Microcystin YR
Midazolam
Midodrine
Minoxidil
Mirtazapine
Mizolastine
Moclobemide
Molinate
Monochloracetate
Monocrotophos
Monolinuron
Monuron
Morphin-3βD-glucuronide-D3
Morphine
Morphine-3-β-D-glucuronide
Myclobutanil
Naled
Naloxone
Nandrolone
Nicardipine
Nicosulfuron
Nicotine
Nifedipine
Nifenazone
Nimodipine
Nisoldipine
Nitenpyram
Nitrazepam
Nitrendipine
Nitrilotriacetic Acid (NTA)
Nivalenol
N-Nitrosodimethylamine (NDMA)
Nodularin
Norbuprenorphine
Nordiazepam
Norfenefrine
Norfentanyl
Normorphine
Nortriptyline
Noscapine
Olanzapine
Omethoate
Ondansetron
Orphenadrine
Oxadixyl
Oxamyl
Oxazepam
Oxcarbazepine
Oxetacaine
Oxitropium
Oxprenolol
Oxycodone
Oxydemeton methyl
Paclobutrazol
Papaverine
Paracetamol
Parathion
Parathion-methyl
Paroxetine
Penconazole
Pencycuron
Pendimethalin
Pentachlorophenol
Pentobarbital
Pentoxyverine
Perazine
Permethrin
Perphenazine
Phenazone
Phencyclidine
Pheniramine
Phenmedipham
Phenthoate
Phenylephrine
Phenyltoloxamine
Phorate
Phorate sulfoxide
Phosalone
Phoxim
Physostigmine
Picoxystrobin
Pilocarpine
Pindolol
Pioglitazone
Pirbuterol
Pirenzepine
Pirimicarb
Pirimiphos-ethyl
Pirimiphos-methyl
Piroxicam
Prajmalium
Prazepam
Prazosin
Primidone
Procainamide
Prochloraz
Progesterone
Promazine
Promecarb
Prometon
Prometryn
Propachlor
Propafenone
Propallylonal
Propamocarb
Propamocarb HCl
Propanil
Propaquizafop
Propargite
Propazine
Propiconazole
Propionylpromazine
Propoxur
Propranolol
Propyphenazone
Propyzamide
Prosulfuron
Prothiopendyl
Protriptyline
Pseudoephedrine
Pyraclostrobin
Pyributicarb
Pyridate
Pyrimethanil
Pyriproxyfen
Quetiapine
Quinapril
Quinine
Quinmerac
Quinoxyfen
Quizalofop-p-ethyl
Ramipril
Ranitidine
Repaglinide
Resmethrine
Rimsulfuron
Risperidone
Ritodrine
Ropivacaine
Rosiglitazon
Scopolamine

Sebuthylazine
Serotonin
Sertindole
Sertraline
Sethoxydim
Siduron
Sildenafil
Simazine
Simetryn
Sotalol
Spinosad A
Spinosad D
Spiroxamin
Sulfotep
Sulindac
Sulpiride
Sulprofos
Talinolol
Tamoxifen
Tebuconazole
Tebufenozide
Tebufenpyrad
Tebuthiuron
Teflubenzuron
Telmisartan
Temazepam
Tepraloxydim
Terbucarb
Terbuthialzine
Terbuthialzine desethyl
Terbuthylazine
Terbutryn
Terfenadine
Testosterone
Tetracaine
Tetrachlorvinphos
Tetraconazole
Tetrahydrocannabinol
Tetrazepam
Tetryzoline
Theobromine
Theophylline
Thiabendazole
Thiacloprid
Thiamethoxam
Thidiazuron
Thiobencarb
Thiodicarb
Thiofanox
Thiofanox-sulfone
Thiophanate methyl
Thioridazine
Thiuram
Tilidine
Timolol
Tizanidine
Tocainide
Tokuthion
Tolbutamide
Toliprolol
Tolylfluanid
Tramadol
Trazodone
Triadiminol
Triallate
Triamcinolone
Triamterene
Triazolam
Triazophos
Trichlorofon
Triclopyr
Trifloxystrobin
Triflumuron
Trifluperazine
Trifluperidol
Trifluralin
Triforine
Trimethoprim
Trimipramine
Urapidil
Valsartan
Vardenafil
Venlafaxine
Verapamil
Vincamine
Vinclozolin
Warfarin
Yohimbine
Zaleplon
Zearalenon
Zolpidem
Zopiclone
Zuclopenthixol