Investigation of the Contamination of Water Resources by Agricultural Chemicals and the Impact on Environmental Health

Volume 1: Risk Assessment of Agricultural Chemicals to Human and Animal Health

Report to the

WATER RESEARCH COMMISSION

Edited by

J.M. Dabrowski

CSIR, Natural Resources and the Environment

In collaboration with

WRC Report No. 1956/1/15
ISBN 978-1-4312-0711-4

October 2015

Obtainable from
Water Research Commission
Private Bag X03
Gezina, 0031

orders@wrc.org.za or download from www.wrc.org.za

This report forms part of a series of two reports. The other report in the series is:

Dabrowski, J.M. (2015): *Investigation of the Contamination of Water Resources by Agricultural Chemicals and the Impact on Environmental Health. Volume 2: Prioritizing human health effects and mapping sources of agricultural pesticides used in South Africa.* WRC Report No. TT 642/15.

A CD with pesticide use maps is enclosed at the back of Volume 2 (the TT report).

EXECUTIVE SUMMARY

BACKGROUND

Agricultural activity is potentially a source of a number of hazardous chemicals in water resources. Concerns have been expressed that some of the pesticides used in agricultural practice (crop spraying and animal disease control) may enter and pollute the rivers and dams and cause endocrine disrupter effects in animals and humans that use the water for drinking and recreational purposes. A scoping study (Burger and Nel, 2008) indicates that there is no clarity on the extent and level of contamination of water resources by agricultural products with ED (endocrine disrupting) properties. However, a number of WRC studies have been done, identifying different chemicals in different areas that are hazardous as well as having ED properties and some studies identified EDCs in water resources and indicated ED effects in sentinel species in and around contaminated water resources.

Most of these studies in South Africa are not specifically focused on the link between the chemicals used in agricultural practices and the impact on human health with water as a pathway. This project will focus specifically on agricultural chemicals. As stated in the scoping study, gaps in knowledge exists and research is necessary which will lead to guidelines for South African authorities to direct the safe use of agricultural chemicals in water resource management.

AIMS and OBJECTIVES

The overall aim of the project is to determine the extent and level of contamination by agricultural chemicals (pesticides, herbicides and plant growth regulants), including Endocrine Disruptive (ED) properties and selected risk assessments for the environment (animal and human health).

This was accomplished by addressing the following specific objectives:

1. To prioritise agricultural chemicals contaminating water for analyses based on hazard (e.g. toxicity and potency);
2. To analyse, *inter alia*, water and sediments for ED activity, e.g. the oestrogenic and anti-androgenic activity; and effects on the immune and thyroid functions, where applicable;
3. To chemically analyse for the active ingredients in samples for presence of specific pesticides by following accredited methods;
4. To assess the human exposure to EDC's in the selected chemicals through, *inter alia*, drinking water, food ingestion, dermal absorption and inhalation by measurement and modelling in selected areas;
5. To identify and assess the sources of agricultural chemicals in water resources for selected areas;
6. To assess the risks caused by agricultural chemicals for animal and human health

This report deals specifically with Objectives 2, 3, 4 and 6. Objectives 1 and 5 are addressed in Volume 2 of this report.

Whilst the title of "agricultural chemicals" includes more than simply pesticides, it was acknowledged that this was a key constituent of concern requiring attention for the project. It was also noted during consultative workshops that, given the number of pesticides registered in South Africa, identification of pesticides for analysis was not only difficult but quantitatively prohibitively expensive, with a screening process thus employed in conjunction with a chemical prioritisation matrix (see Volume 2 of this report) in which the presence of over 300 pesticides was determined in predominantly surface waters and sediment. In conjunction with this, the use of several bioassays developed for the WRC Endocrine Disrupting Chemical Project focusing on reproductive endpoints was also conducted.

SITE SELECTION

Three study areas were selected following a consultative approach. These were: the Letsitele catchment in the vicinity of Tzaneen – an area dominated by tropical and citrus fruit production; the Lomati catchment which drains into the Komati River in the vicinity of Komatipoort (dominated by sugar cane production as well as by other tropical and citrus fruits); and the Vals and Renoster catchments in the Free State, both of which enter the Vaal River, in the vicinity of Kroonstad and Viljoenskroon – an area of intensive maize production. The project initially conducted sampling for ED bioassays and organic and inorganic constituents from surface water resources and sediments in these three selected sites. It was recognized that impacts on groundwater were also potentially significant and this was later included.

MONITORING WATER AND SEDIMENT FOR PESTICIDES

Water and sediment samples were analysed for pesticides by LiquidTech using LC/MS/MS techniques. A qualitative screening approach in combination with a pesticide prioritisation matrix was used to identify target pesticides for further quantitative analysis (see Volume 2 of the report). Screening techniques analysed for the presence of in excess of 200 active ingredients. A wide variety of pesticides were detected within all study areas:

- Diphenylamine, imizalil, thiabendazole, imidacloprid and propiconzaole were quantitatively analysed in water and sediment samples collected from the Letsitele catchment. Concentrations of all pesticides in water and sediment were typically very low (no pesticide was detected above 0.06 ug/L) in comparison to concentrations of other pesticides detected in the other two study areas. Thiabendazole and imidacloprid were detected most frequently. These are both applied to citrus. Thiabendazole was detected at very low concentrations, which is likely attributable to its low rate of application and its low mobility in the environment. Imidacloprid is applied at higher rates and is highly mobile in the environments and was detected at comparatively higher concentrations.

Additionally, imidacloprid was detected at relatively higher concentrations in borehole samples collected from the area, indicating its tendency to leach as a result of its physicochemical properties.

- Alachlor, atrazine, imidacloprid, simazine and terbuthylazine were quantitatively analysed in water and sediment samples collected from the Vals and Renoster river catchments. Atrazine and terbuthylazine are highly mobile pesticides and were detected in all water samples collected from all sites at concentrations that were higher than for all other pesticides measured in this study. Both of these herbicides are used in high quantities on maize, which was the dominant crop in the study area. The fact that these herbicides were detected constantly throughout the year (during the wet and dry season), indicates that they have essentially saturated the hydrological cycle and further detailed monitoring surveys (of surface and groundwater) targeting these specific herbicides would be warranted in the future. Simazine was also detected at relatively high concentrations, although not as frequently as the aforementioned herbicides. While this herbicide is also regarded as highly mobile, it is used in comparatively lower quantities than atrazine and terbuthylazine. Alachlor was detected in only three samples collected from the study area. A large number of additional pesticides were detected in the qualitative screens. Some of these are registered for agricultural use (e.g. metolachlor, terbutryn, ametryn, carbendazim, thiabendazole), while others are used to control bush encroachment (e.g. tebuthiuron and prometon).
- Atrazine, imidacloprid, terbuthylazine, thiabendazole and carbofuran were quantitatively analysed in water and sediment samples collected from the Lomati River catchment. Of these pesticides, all, except for thiabendazole, were detected relatively frequently. All four pesticides are regarded as being highly mobile in the environment, while thiabendazole has very low mobility and is applied at low rates of application. In addition, hexazinone, diuron and ametryn, all of which have medium to high mobility, were frequently detected samples submitted for qualitative screening. Atrazine and terbuthylazine were detected at comparatively lower concentrations than in the Vals and Renoster rivers. Samples collected from a water purification works in Langloop had very similar concentrations of pesticides to those detected in the river from which water was sourced (Lomati River), indicating that the treatment technology had very little effect on removing pesticides from drinking water.
- Contamination patterns for each of the pesticides detected in the study area were generally well predicted by an index of mobility (GUS index), with high and medium mobility pesticides being detected either more frequently or at higher concentrations than those in the low mobility category.
- In general, for all pesticides detected in all sites, the prioritization matrix developed in Volume 2 of this report provided a valuable indication of pesticides likely to be present in water resources adjacent to specific crop types. Mobility indices were derived using physicochemical data not derived under South African conditions. In spite of this, available physicochemical data from international databases provided an accurate indication of mobility under South African conditions.

MONITORING AIR FOR PESTICIDES

Spray drift of pesticides may result from the movement of vapour or particles away from the target area by the action of air flow, which may lead to contamination of the surrounding natural resources. From a health risk perspective, spray drift can result in exposure via contamination of adjacent water bodies as well as via inhalation during pesticide application. There are a number of factors that affect the extent of drift including meteorological conditions, application parameters, and the physical-chemical properties of the spray solution. Monitoring environmental exposure during and after spray operations is important in assessing possible risks but is challenging from a number of perspectives, including the need for specialised sampling equipment, extraction and analysis techniques as well as the logistics involved in designing and setting up a field study that accommodates a farmer's plans under unpredictable weather conditions. The use of computer models that simulate airborne concentrations and off-site deposition of pesticides originating from spray drift is therefore an attractive and practical alternative approach. It is however important to validate such models with measured data to assess their reliability in generating exposure data for risk assessment purposes.

The aim of this chapter was therefore to validate an air dispersion model which could potentially be used to predict spray drift for human health risk assessments. This was achieved by firstly monitoring pesticide spray drift during pesticide application at a large scale agricultural farm. This required that a method to sample and quantify the spray drift of a widely used pesticide, namely atrazine, be developed and validated. Secondly, spray application data collected during the sampling campaign was used as input data to the AGricultural DISPersal (AGDISP) model to generate predicted airborne concentrations and deposition quantities of pesticide. Finally, the predicted model simulations were compared to measured data to determine the accuracy of the model.

Spray drift monitoring was conducted during spraying of weeds in a sorghum field in Standerton, South Africa, under calm meteorological conditions. The herbicide solution contained atrazine (which was targeted as a drift tracer) and was applied using a boom sprayer. Both deposition and airborne spray drift samples were collected downwind of the application area using chromatography paper fallout sheets and PUF high volume samplers respectively. Additionally, data required by the AGDISP model as input for drift simulations was obtained from the farmer. Meteorological information was measured using a portable weather station and the droplet size distribution (DSD) was determined using magnesium oxide coated slides. Three different sets of droplet distributions were thus obtained.

The PUF samples were extracted using a plunger technique with a mixture of hexane and acetone and the extracts were analyzed for atrazine using a gas chromatography-nitrogen phosphorus detector (GC-NPD) based method. The deposition samples on the other hand were extracted with the same solvent mixture but using an ultrasonic extraction technique and were later analyzed with both GC-NPD and direct sample analysis-time of flight mass spectrometry (DSA-TOFMS).

To determine the important input factors of the model that significantly affect spray drift predictions, sensitivity tests were conducted using ranges based on maximum and minimum values recorded during the sampling period. Finally, the model was used to simulate spray drift for different application scenarios for the study areas of interest.

- The active pesticide ingredient, namely atrazine, was successfully used to monitor downwind spray drift under local conditions during application due to its stability and compatibility with the instrumentation that was used.
- A good correlation between model predicted and field experimental results was obtained for airborne levels which suggests that the model can be used to provide a good estimate for airborne drift in risk assessment studies or for regulatory purposes.
- Compared to the experimental results, the model under-predicted the deposition by up to one order of magnitude compared to the GC-NPD results and even more compared to the DSA-TOFMS results close to the field.
- Sensitivity studies showed that the model was strongly dependent on droplet size as supported by other studies. Among the meteorological parameters, wind speed was the most significant factor.
- This study has, for the first time, validated the AGDISP model under South African conditions using the pesticide active ingredient up to 400 m from the application site.

ANDROGENIC ACTIVITY

Water and sediment from the three selected catchments were extracted and screened for (anti-)androgen activity. The specific mechanism of action that was quantified was the binding to – and consequent activation and/or inhibition – of the cytoplasmic androgen receptor. It is important to note that it was specifically receptor binding that was investigated. Furthermore, the extraction methods to concentrate the receptor ligands from the environmental matrices targeted organic chemical pollutants, that had mostly apolar (sediment adsorbed compounds) and slightly polar characteristics (water soluble compounds). This means that metal ions and inorganic salt ions for example would have been excluded from this analysis.

A human breast cancer cell line that had been genetically modified to also contain and express the firefly luciferase enzyme was used as the biological measuring instrument. In the case of successful binding of ligands to the androgen receptor, leading to expression of luciferase, the subsequent amount of light emitted by the cells is directly proportional to the amount of pollutant ligands the cells were exposed to. This signified activation of the receptor. Inhibition of the receptor was determined by quantifying the decrease of light in cells already excited with a known concentration of testosterone. In the case of androgen receptor activation, the amount of light was quantified in terms of the amount of light that was emitted by a known concentration of testosterone (the positive control) and expressed as bio-assay equivalents. Inhibition was quantified in terms of how many times the light

signal was dimmed in relation to the light signal off of the cells after exposure to a known concentration testosterone, i.e. fold-inhibition.

Although (anti-)androgen activity was observed in all three the catchments, there was no distinct pattern over the duration of the study, except for the fact that the most (anti-)androgen activity was found in the sediment samples from the Vals and Renoster catchment, and also clear evidence of cytotoxicity. This lack of corresponding bioassay response with the chemistry data emphasises the fact that knowing the exact concentration of pollutants does not predict their effects on wildlife living in that environment. The bioassay results include the synergistic response of the compound mixture found in the environment which includes enhancing the effect, but often limiting the effect because of compounds competing for binding to the receptor.

Bioassays to inform on environmental status are a useful tool but should be used in conjunction with instrumental analysis and a clear understanding of the mechanism of action the bioassay is based on.

OESTROGENIC ACTIVITY

In-vitro bio-assays are widely used as screens to determine if specific compounds or environmental samples, for example water or sediment, have endocrine disrupting activity. In this study the recombinant yeast oestrogen screen (YES), as well as the T47D-KBluc reporter gene assays were used to assess oestrogenic activity in the water and sediment samples from the selected agricultural areas in South Africa.

Oestrogenic activity was detected in all catchment areas in the T47D-KBluc assay. In the Letsitele catchment, oestrogenic activity ranged between below the detection limit to 0.58 ng/L for water and below detection limit to 14.68 ng/kg for sediment samples. In the Lomati catchment, oestrogenic activity ranged between below the detection limit to 0.42 ng/L for water and below detection limit to 65.04 ng/kg for sediment samples. In the Vals and Renoster catchment, oestrogenic activity ranged between below the detection limit to 6.78 ng/L for water and below detection limit to 254.95 ng/kg for sediment samples. Anti-oestrogenic activity was detected in VL3 water samples in July and October 2012. In the additional borehole samples from 2013, oestrogenic activity ranged between below the detection limit to 0.35 ng/L.

Oestrogenic activity was detected in water and sediment samples from all three study areas. In general, sediment samples had higher estradiol equivalents (EEq) compared to the water samples which may indicate bioaccumulation of oestrogenic compounds in the sediment.

The Vals and Renoster catchment seemed to have the highest oestrogenic activity in the water samples with five above the recommended drinking water trigger value of 0.7 ng/L Eeq (RN4-Oct 12; VL2-Jul 12; VL2-Jan 13; VL2-Apr 13; VL3-Jan 13). Cytotoxicity was also detected at the abovementioned sites. Further investigation of these sites is therefore recommended.

It should be kept in mind that water and sediment samples consist of a complex mixture of chemicals with possible (anti)-androgenic and (anti)-oestrogenic activity, as well as other chemicals not measured, that could affect the outcome of the assay. Investigating the area surrounding the sites and the data from the chemical analysis of the water samples might give some valuable information to identify possible sources of oestrogenic contamination at the sites.

ANIMAL HEALTH ASSESSMENT

Bioassays and screening tests were part of a fundamental approach to hazard and risk assessment and in order to determine possible adverse effects on human and animal health, some clinical observations were required. Although it was not within the scope of the project to address cause and effect relationships or conduct public health investigations, it was acknowledged that due to the complexity of concurrent exposure to multiple potentially hazardous constituents, the use of animals for sentinel monitoring purposes was required in order to be able to arrive at some defensible statement on the potential impacts of exposure to agricultural chemicals in the sites investigated.

In recognition of the limitations of interpretation of bioassays, and other endpoint complexities, poultry (broilers) were used to obtain tissue samples for histopathological and clinical biochemical investigation. Primary advantages for including sentinel observations are the significant reduction in exposure variation when compared to public health investigations, and statistical confidence in terms of the high number of sample replicates and repetitions. To further this advantage, the use of commercial specimens (broilers from commercial production units in the selected study areas) as opposed to those obtained in the rural communities in the study areas emerged as a preferred sample source. Additional clinical observations in which a porcine (pig) model was used are also presented, this benefit largely related to the additional use thereof for biomedical research and corresponding correlations to human health. The key findings were:

- Inorganic water quality constituents may represent a fundamentally greater potential hazard to various water users compared to organic chemicals. This is largely due to the low concentrations of organic chemicals observed and the intermittent exposure to airborne pollutants. This is also influenced by the combination of naturally occurring inorganic constituents and those sourced from agricultural activities.
- Commercial production animals present a viable low-cost means of obtaining representative clinical observations which can be used to determine if further human health investigations are indicated in a site-specific exposure scenario.
- Differentiating between inorganic constituents from naturally occurring geochemistry and those from agricultural chemicals is required to effectively formulate management objectives.

- A fundamental source, pathway and receptor approach is required in order to determine the potential hazards posed by, and safe use of, agricultural chemicals.
- For organic chemicals it also follows that site-specific factors, in terms of nutritional status, the presence of sensitive user groups, within the total exposures relevant, may be deterministic in terms of potential hazards posed to animals and humans. The concentration present in the exposure pathway may thus serve as more of an indication of the presence of a potential hazard and less of a key guideline value.
- For inorganic chemicals site-specific factors are also relevant, but the increased concentration ranges observed may imply that concentration alone plays a more significant role.
- In terms of confined animal feeding operations the dual use of anaerobic digestion and a separator stage may assist in reducing the adverse effects linked to health parameters in receiving receptors. This is a growing and complex issue which also warrants further research.
- Further investigation is needed to identify the sources of bromide, both from naturally occurring geochemical anomalies and agricultural chemicals.
    - Significant increases have been noted when various disinfection chemicals have been used in drinking water applications for both surface and groundwater in agricultural water use activities.
    - Further investigation is indicated to establish the key adverse effects linked to bromide. Whilst the thyroid disruption and dysfunction appears to be a consistent observation in broilers and porcine sentinel monitoring, additional reproductive and induced deficiencies require more focused research.
    - Concerns for increased environmental bromide levels and consequently more brominated disinfection by-products due to industrial discharges, notably from coal-fired power stations with various emission control treatments, are recognised internationally as requiring increased monitoring and regulation.
- The inclusion of the full ICP-MS suite of chemicals, with the notable inclusion of bromide, for both water resource compliance monitoring, and the supporting tissue tests as conducted for the sentinel monitoring, is justified and should be part of compliance monitoring for Section 21 activities of the National Water Act.
- In the interim a position statement for competent authorities currently administering both applicable sections of the NEMA and NWA should be prepared and submitted as an outcome of this project.

HUMAN HEALTH RISK ASSESSMENT

A human health risk assessment of pesticides detected in water and sediment samples collected from sampling sites in each of the three study areas was conducted. The methodology with which the human health risk assessment was

carried out is described by the US Environmental Protection Agency (US EPA) and the World Health Organization (WHO). For non-carcinogenic toxic effects of organic pollutants, a Hazard Quotient (HQ) was calculated, comparing the expected exposure to the pesticide to an exposure that is assumed to not be associated with toxic effects. For human oral exposure, the Average Daily Dose (ADD) related to consumption of contaminated water, meat, milk, fish and plants was estimated from the measured concentrations and was compared to a Reference Dose (RfD) as reported by the EPA. A HQ less than 1 was considered to be safe for a lifetime of exposure. A risk estimate represents the theoretical excess cancer risk and is regarded as the risk of developing cancer in addition to the background cancer incidence. The WHO defines the acceptable risk level as “an estimated upper-bound excess lifetime cancer risk of one additional cancer per 100 000 of the population exposed to the substance for 70 years (life expectancy)”.

Different exposure scenarios were used to predict possible human health risks, including a person making use of the surface water for their domestic use, a farmer using the water to irrigate produce, a person regularly eating fish from the area and a person making use of the water for recreational activities such as swimming. Modelled air distributions and depositions were also used to calculate potential risks based on spray-drift of applied pesticides.

- Based on ingestion of water and food, both HQs and risks of developing cancer were many orders of magnitude lower than what is considered safe or acceptable for each of the three study areas.
- Risks of developing cancer were predicted at very low levels as a result of exposure to atrazine in the Lomati catchment.
- Samples collected from the Vals and Renoster rivers contained simazine, atrazine and alachlor which were responsible for predicted cancer effects at levels lower than the excess cancer risk considered as acceptable. HQs were many orders of magnitudes lower than ‘1’ which is considered safe for a lifetime exposure.
- HQs of samples collected from the Letsitele catchment were three orders of magnitude lower than the allowable exposure (or 1/1000th of the safe dose) and no cancer risks were predicted.
- HQs of samples collected from the Lomati catchment were close to 1/100th of that considered safe with cancer risks 1000 times less than an acceptable risk.
- HQs of samples collected from the Vals and Renoster rivers were the highest, with hazard quotients close to 50 times lower than the safe level and cancer risks slightly lower than the 1 in 100 000 acceptable risk.
- Based on inhalation of pesticides, modelled air concentrations illustrated that of the three pesticides included in the assessment, alachor presented a higher risk than atrazine and simazine, but was within Occupational Health safety levels (ACGIH®) with HQs ranging from 1 to 20% of the safe threshold values. These values are not used to protect public health, however, as they do not typically take into account sensitive individuals.

- Average estradiol equivalent quantity (EEq) values for the Letsitele and Lomati catchments were less than the trigger value of 0.7 ng/L. Values for samples collected from the Vals and Renoster rivers indicate that more detailed EDC testing is required.

CONCLUSIONS

Broad conclusions relevant to the overall objectives of the study are given here. More task-specific conclusions are provided in relevant chapters contained in the report.

- The use of bioassays, ICP-MS for trace elements and pesticide screening tools, developed for the WRC EDC programme and this project, are by implication required observations for elucidating on ED effects associated with exposure to agricultural chemicals.
- A number of different pesticides were regularly detected at sites in the three different study areas chosen for this study. Of the pesticides analysed in this study, the herbicides atrazine, terbuthylazine and simazine and the insecticide imidacloprid were frequently detected in surface and ground water samples at relatively higher concentrations in comparison to other pesticides.
- The occurrence of pesticides in water resources could largely be explained by patterns of use as well as physicochemical properties of pesticides applied to crops in the study areas. This indicates that the pesticide use data and international databases of pesticide physicochemical properties are reliable indicators of pesticide behaviour of under South African conditions.
- The monitoring programme implemented in this study allowed for a survey of a wide number of agricultural chemicals applied in three distinct agricultural crop settings. A disadvantage of this approach was that it sacrificed a high temporal resolution of contamination, resulting in high uncertainty related to exposure profiles for all pesticides. Critical periods of peak contamination during rainfall events and spray application were therefore not captured and as a result, concentrations reported here are most likely not representative of maximum peak concentrations.
- A good correlation between AGDISP models predictions and measured airborne concentrations suggests that the model can be used to provide a good estimate for airborne drift in risk assessment studies or for regulatory purposes. Results from this and other modelling studies strongly support the use of such models in regulatory and environmental health risk assessments in South Africa.
- In order to interpret the hazards and risks posed by agricultural chemicals, background baseline exposure to inorganic chemistry is required as a starting point. Failure to include this results in less confidence in interpreting both the bioassay results that may be obtained and exposure assessments. Use of this water quality data is required in order to meaningfully interpret the context of hazards posed by organics and inorganics, without which a differential diagnosis may be difficult to reach. This is presented in greater detail in the Monitoring and Assessment Volume of the WRC Manual on EDCs (WRC Report TT 612/14).

- Limitations of *in vitro* assays adopted in this study include the inability to detect compounds that are pro-oestrogens (compounds that are converted to oestrogens *in vivo*) or to conclusively identify the causative chemical without chemical analysis. Despite the rigorous sampling approach adopted in this study, based on the patterns of contamination observed, it was not possible to identify agricultural chemicals responsible for causative effect.
- The assays do however detect chemicals based on their biological activity and determine the total oestrogenic or androgenic content of a given sample. Significant responses in an *in vitro* bioassay should therefore be used as an indicator for further investigation using *in vivo* test models, and/or identification of the active chemical. A negative response would suggest that further investigations might not be warranted. Bioassays therefore play a major role as initial screening tools for environmental samples. In this respect, a literature review of ED responses to exposure to pesticides detected in this study indicates that pesticides detected in samples collected from the study areas may have contributed to the observed ED activity.
- In this respect, the comparatively higher EEq values in combination with the ubiquitous presence of atrazine, simazine and terbuthlyazine in water samples collected from the Vals and Renoster catchments, highlights this area as a priority for further research.
- The inclusion of the full ICP-MS suite of chemicals, with the notable inclusion of bromide, for both water resource compliance monitoring, and the supporting tissue tests as conducted for the sentinel monitoring, is justified and should be part of compliance monitoring for Section 21 activities of the National Water Act.
- Overall, the predicted adverse human health (cancer and toxicity) effects based on exposure to the pesticides detected in water samples collected from all study areas were considered to be low. Within the context of this study, exposure to pesticides via air presents a greater risk with possible adverse health effects for the time of pesticide application and as a result of the deposition of pesticides in the surrounding area.
- The low hazard noted for pesticides in the selected study areas, may be as a function of the hazard itself being transient in nature. It may be argued that the surface water recharge in the selected sites reduced the potential for risk, but this may not be true for other areas or groundwater, and these should accordingly be a focus point of any site-specific investigation. An increased frequency of observations is ideally required for the bioassays and suspected organic hazards (e.g. pesticides) in these priority areas (e.g. Vals and Renoster river catchments).
- Despite the low hazards posed by pesticides detected in this study, the increased recognition of ED effects from exposure to low concentrations and complexities around concurrent exposure to multiple potential hazards via the water pathway, a precautionary approach is still justified.

RECOMMENDATIONS FOR FUTURE RESEARCH

The key research themes requiring elucidation include:

- Decisions related to monitoring of pesticides in the selected study areas benefitted significantly from the pesticide use data, prioritization matrix and pesticide use maps developed in this project (refer to Volume 2 of this report). It is recommended that these resources be consulted when undertaking similar studies in the future. It is further recommended that regular updates of pesticide use data, spatial crop distribution and associated pesticide use maps are produced and disseminated to ensure the availability of up to date information for use in design of monitoring programmes and risk assessment studies.
- From a monitoring perspective, further research should focus on the development and use of passive samplers (including biomonitors) in providing time integrated measurements of pesticide contamination. While these samplers have historically been used for monitoring more hydrophobic organic chemicals (e.g. organochlorines), with advances in materials and analytical techniques, there is an increasing body of literature that demonstrates their application in monitoring current use pesticides representing a wider range of chemical classes.
- While the analytical approach adopted in this study catered for a large number of different pesticides, it is important to note that glyphosate (the most heavily applied pesticide in the country) was not included in screening or quantitative analysis. Considering its high quantity of use as well as increasing evidence of human health-related effects, future research should focus on developing analytical methods for detection of this pesticide (and its breakdown products) in water resources in South Africa.
- Given the typically transient nature of pesticide contamination in water resources, the consistent detection of atrazine and terbuthylazine at study sites in the Free State indicates a saturation of the water resource, to the extent that a more detailed monitoring programme, with a higher frequency of sample collection (or more sites) is warranted so as to establish a more accurate picture of exposure associated risks.
- Data on physicochemical properties of pesticides in South African environmental conditions are not available. International databases were therefore consulted in order to obtain the relevant data for calculation of the mobility (GUS) index. Studies have shown that physicochemical properties of pesticides can vary geographically, depending on local climatic and soil conditions. Considering this limitation however, the occurrence of pesticides detected in water resources in this (and other) studies was well predicted by physicochemical data available in international databases. The fact that these originate from international databases should therefore not be seen as a reason for not relying on this data for future modelling approaches undertaken in South Africa.
- The risk a pesticide poses to human health (and the aquatic environment) is dependent on a number of factors, including the relative toxicity of the chemical, the relative mobility (as influenced by physicochemical properties), recommended application rates (i.e. quantity of use) and agricultural practices (e.g. correct use

of nozzles). As farmers almost always have a choice of different chemicals to target a specific pest on a specific crop, it is recommended that a manual providing guidelines on choosing agricultural chemicals that minimise effects in non-target environments (both human and ecological health) be produced.

- The use of sentinel monitoring yielded valuable information towards the site-specific animal health assessment. The observations would suggest that further studies into human health with bromide as a key priority chemical are indicated.
- In order to manage agricultural chemicals effectively, it should be noted that the source may be varied to a great extent and include constituents included in animal feeds, washing, sanitizing and disinfection chemicals. It follows that in order to effectively comply with the objectives of the applicable legislation, the beneficial reuse of wastewater can only be conducted if the appropriate monitoring thereof is conducted. It was an observation during this project that many authorizations and licences granted failed to include the constituents relevant to the process involved. The inclusion of all the relevant constituents that are linked to the agricultural activity in question must be included in any licence or authorization granted. Establishing these lists for organic and inorganic constituents remains a fundamental research priority that will allow for appropriate monitoring, assessment and thus management thereof.
- It is also acknowledged that a revision of the 1996 South African Water Quality Guidelines is underway with the irrigation volume being addressed first. It is argued that both Domestic and Animal Watering sections also urgently require revision to align with risk-based approaches that are necessary to appropriately assess and manage the hazards and risks present.
- Given the challenges related to monitoring (due to the transient nature of contamination) and that pesticide contamination in water resources occurs primarily as a result of nonpoint sources (e.g. runoff and leaching), further research should focus on modelling techniques aimed at assessing the fate, transport and mitigation/management options of pesticides in water at multiple scales (field to catchment).
- Furthermore research should focus on the integration of these models into the risk assessment process conducted during the registration of pesticides. While the registration process considers the toxicity of a pesticide, there are no exposure assessment procedures performed to assess the environmental fate and predicted environmental concentrations under South African conditions. Without an assessment of exposure it is difficult to assess the anticipated risk of pesticides in the environment and to impose restrictions on use (i.e. in terms of application rates or geographical areas where use of a pesticide should be restricted or avoided). This will ensure a more pro-active approach to managing pesticides in South Africa as opposed to a reactive approach where risks are effectively only determined after authorization of a product through ad-hoc studies (such as this one). The accurate results achieved using the AGDISP model in this study justify further research into evaluating the use of this and other runoff and leaching models for use in pesticide risk assessment studies in South Africa.

- Given the relatively low ED risks associated with agricultural chemicals in this study, comparative studies comparing the ED effects associated with different crop types, land uses and other important point sources (e.g. mining, industrial and sewage effluent) are encouraged so as to provide improved perspective of the relative importance of these different sources on ED effects.

CAPACITY BUILDING

In total 4 Masters students contributed directly to the objectives of this project, three of which were involved in the research presented in this volume. Of these three, one student graduated in 2013, one has submitted his dissertation during 2015 and the other is ongoing and will likely submit in 2016. Apart from these specific students a number of staff and students in the Environmental Chemical Pollution and Health Research Unit (University of Pretoria) were developed through their participation in conducting oestrogenic bioassays (including sample preparation and extraction, cell culture and media preparation and YES and T47D-KBluc bioassay methods). Full details of capacity building can be viewed in the Appendix to this report.

KNOWLEDGE DISSEMINATION

Three papers have been submitted and accepted in national and international peer reviewed journals, of which one paper originates from research documented in Chapter 4 of this report. This paper was accepted in the international environmental science journal, (3.34 Impact Factor). This is the first study that has documented the use of the AGDISP model in South Africa and one of only a few world-wide that have validated the model through field-based measurements. See Volume 2 of this report for further details on other papers published from this research. In addition, numerous presentations of research conducted during this project have been presented at both national and international conferences held during the course of this project. Full details of knowledge dissemination can be viewed in the Appendix to this report.

ACKNOWLEDGEMENTS

This project was initiated by the Water Research Commission (WRC) of South Africa through a directed call and publication of associated terms of reference. The CSIR together with collaborators from the University of Pretoria and North West University responded to the call and carried out the research to meet the objectives stipulated in the terms of reference. The project was managed by the WRC.

The authors of this report acknowledge inputs from the Reference Group which significantly contributed to supporting and guiding the project team. The Reference Group was comprised of the following members:

Ms A Moolman	*Chairperson (2010-2011)*
Dr GR Backeberg	*Chairsperson (2012-2013)*
Dr S Mpandeli	*Chairperson (2014-2015)*
Dr SH Jooste	*Department of Water and Sanitation*
Prof NH Casey	*University of Pretoria*
Mr TR Nepfumbada	*Department of Agriculture, Forestry and Fisheries*
Mrs B Genthe	*CSIR*
Mr E van der Walt	*Agricultural Research Council*

In addition, the various landowners in the Letsitele, Lomati, Vals and Renoster catchments are acknowledged for allowing access to their property for the purposes of collecting samples.

Etienne van der Walt, Pietie van Niekerk and Annette Venter from the Agricultural Research Council (ARC) are sincerely thanked for allowing members of the project team to use their sampling equipment, for their active involvement and assistance during sampling, and for offering to assist with sample analysis in their laboratories. We are grateful to Ernst Bekker who also assisted with air sampling. We thank the Blignaut family in Standerton, particularly Sias, Peet, Hennie and Annette for allowing us to use their farm to carry out a detailed spray drift sampling campaign and for providing valuable operational information that was used as input for modelling purposes.

Perkin Elmer is thanked for providing access to the AxION DSA-TOFMS instrumentation.

In addition the following individuals are acknowledged:

Prof N Duncan	*University of Pretoria (Pathology)*
Dr Gabre Kemp	*University of the Free State (Pesticide Analysis)*
Mr P Rankin	*GfK Kynetec (Pesticide Use Data)*

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CHAPTER 1: INTRODUCTION

by

J.M. Dabrowski[1], N.H. Aneck-Hahn[2], P.B.C. Forbes[3], B. Genthe[4], R. Pieters[5], J.A. Meyer[6]

[1]*CSIR, Natural Resources and Environment, PO Box 395, Pretoria, 0001*

[2] *Environmental Chemical Pollution and Health Research Unit, Faculty of Health Sciences, University of Pretoria, PO Box 667, Pretoria 0001*

[3]*Department of Chemistry, Faculty of Natural & Agricultural Sciences, University of Pretoria, Pretoria, 0002*

[3]*CSIR, Natural Resources and Environment, PO Box 320, Stellenbosch, 7599*

[5] *Unit of Environmental Sciences and Management; North-West University Potchefstroom; 2520*

[6]*PO Box 2312, Faerie Glen, Pretoria*

1.1 PROJECT BACKGROUND

Agricultural activity is potentially a source of a number of hazardous chemicals in water resources. Concerns have been expressed that some of the pesticides used in agricultural practice (crop spraying and animal disease control) may enter and pollute the rivers and dams and cause endocrine disrupter effects in animals and humans that use the water for drinking and recreational purposes. A scoping study by Burger and Nel (2008) indicated that a large number of pesticides with endocrine disrupting (ED) properties are registered for use in all the Water Management Areas (WMAs) of the country. The study however found that that there is no clarity on the extent and level of contamination of water resources by agricultural products with ED properties. While a number of WRC studies have identified different pesticides in different areas (London *et al.*, 2000; Sereda and Meinhardt, 2003; Dabrowski *et al.*, 2011) including those that are hazardous and have ED properties with evidence of ED effects in sentinel species in and around contaminated water resources (Bornman *et al*., 2007), few studies have linked agricultural chemical use to human and animal health effects (including ED effects) via the water pathway.

As such, the overall aim of this project was to perform a comprehensive study on the extent and level of contamination of selected water resources in South Africa by agricultural chemicals (pesticides, herbicides and plant growth regulants) and their risk towards animal and human health in terms of their toxicity and ED potential.

This was accomplished by addressing the following specific objectives:

1. To prioritise agricultural chemicals contaminating water for analyses based on hazard (e.g. toxicity and potency);
2. To analyse, *inter alia*, water and sediments for ED activity, e.g. the oestrogenic and anti-androgenic activity; and effects on the immune and thyroid functions, where applicable;
3. To chemically analyse for the active ingredients in samples for presence of specific pesticides by following accredited methods;
4. To assess the human exposure to EDC's in the selected chemicals through, *inter alia*, drinking water, food ingestion, dermal absorption and inhalation by measurement and modelling in selected areas;
5. To identify and assess the sources of agricultural chemicals in water resources for selected areas;
6. To assess the risks caused by agricultural chemicals for animal and human health

1.2 PROJECT SCOPE AND EXTENT

The duration of the project was from April 2010 to March 2015. The proposed project focuses on human and animal health; although aquatic ecological risk assessment is not disregarded as unimportant, it is specifically excluded from the focus of this project. It is furthermore recognised that agricultural chemicals used in malaria control agents, industrial and mining spraying to control weeds,etc. and chemicals not related to agriculture but from other human related activities such as pollution from sewage waste plants might also contribute to ED effects that are present in water resources. This project focusses specifically on agricultural chemicals, including pesticides, fertilizers and any agricultural stock remedies used in agriculture.

1.3 CONTRIBUTION OF INDIVIDUAL CHAPTERS TO THE OBJECTIVES OF THE PROJECT

The project was structured into three, related tasks:

1. Field based monitoring and risk assessment of agricultural chemicals in suitable study sites (Objectives 2-6).
2. Prioritisation of risk of agricultural chemicals to human health, based on their quantity of use, toxicity and environmental mobility (Objective 1).
3. Identification of the source of agricultural chemicals through the development of pesticide use maps for South Africa, based on crop distribution and pesticide use data (Objective 5).

This report documents the research conducted in Task 1 of the project with relevant cross-references to outputs associated with Tasks 2 and 3, where applicable. This section details the contribution of individual chapters to Task 1 of the project:

1.3.1 Chapter 2

This chapter deals with the rationale of how study areas (catchments) were selected and provides information on each of the study areas as well as the location of monitoring points located within the study area. Study sites are not described in detail in subsequent chapters and this chapter should always be referred to for study area and monitoring site descriptions and locations. This chapter also provides details on what samples (i.e. water and/or sediment) were collected at each site and what analyses were performed (i.e. quantitative and/or qualitative pesticide analysis, inorganic chemistry, ED bioassays etc.)

1.3.2 Chapter 3

Chapter 3 provides data on pesticide levels detected in water and sediment at all sampling sites in all study areas. Links are made between observed pesticide concentrations and the prioritization tool developed in Task 2 of the project as a means of determining whether pesticide use data is a reliable indicator of what is actually observed in sampling media. Details on sampling methodology and pesticide analysis are provided. This chapter addresses objective 3.

1.3.3 Chapter 4

This chapter evaluates the use of the AGDISP model to estimate off-site (i.e. areas adjacent to agricultural fields) airborne pesticide concentrations and ground deposition resulting from spray drift that occurs during pesticide application. Model simulations were compared with field samples collected through the implementation of a rigorous sampling design. In this manner the accuracy of the model in predicting off-target spray drift was assessed. Results indicate promising application of the model for estimating pesticide exposure from spray drift in South Africa. This chapter contributes to objective 3.

1.3.4 Chapter 5

In chapter 5, results of the screening bioassays for androgenic activation and inhibition from water and sediment samples collected in study areas and sampling sites are presented. The androgen screening bioassay that was used in this study is the MDA-kb2 reporter gene bioassay developed by the US EPA. These assays were performed on samples collected at the same time as those collected for analysis of pesticides and inorganic constituents, allowing for the possibility to describe relationships between the presence of know endocrine disruptors and androgen response. This chapter addresses objective 2.

1.3.5 Chapter 6

In chapter 6, results of the bioassay used to screen for oestrogenic endocrine disrupting activity are presented. This study used the recombinant yeast oestrogen screen (YES) and the T47D-KBluc reporter gene assay. These assays were also performed on samples collected at the same time as those collected for analysis of pesticides and inorganic constituents, allowing for the possibility to describe

relationships between the presence of know endocrine disruptors and oestrogen response. This chapter also addresses objective 2 of the project.

1.3.6 Chapter 7

This chapter shows results of a Principal Component Analysis (PCA) aimed at attempting to determine whether any links could be established between known EDC chemicals (organic and inorganic) and ED bioassay results.

1.3.7 Chapter 8

Agricultural chemicals are not limited to pesticides and this chapter presents data and risk associated with inorganic constituents sampled during the course of the study. Many inorganics have been implicated in endocrine disruption and are therefore important to consider in establishing baseline conditions in order to assist with the hazard and risk assessment procedures formulated by additional sampling techniques generally related to agricultural chemicals, pesticides and potential endocrine disrupting effects and other potential adverse effects. This chapter included an assessment of the fitness for use for recognized water users, specifically livestock and domestic use and therefore contributes to objectives 4 and 6.

1.3.8 Chapter 9

Chapter 9 integrates pesticide and EDC bioassay data collected over the course of the project (water, sediment and air) together with estimates of typical exposure patterns (e.g. consumption of drinking water, dermal adsorption through bathing and swimming etc.) to estimate the human health risk associated with consumption or exposure to pesticides detected in samples collected from all the study areas. This chapter addresses objectives 3 and 6 of the terms of reference.

1.4 AGRICULTURAL CHEMICALS

1.4.1 Act 36 of 1947

South African legislation (Act 36 of 1947) categorises agricultural chemicals into four groups namely; (1) fertilisers, (2) agricultural remedies, (3) farm feeds and (4) stock remedies. The registration and use of agricultural chemicals is under the control of Act 36 of 1947. Agricultural remedies are biological or chemical agents used individually or in mixture to destruct, control, repel, attract or prevent any undesired microbe, alga, nematode, fungus, insect, plant, vertebrate, invertebrate, or any product thereof. Agricultural remedies also include plant growth-regulators, defoliants, desiccants or legume innoculants. Farm feeds are substances possessing nutritional value used to feed domestic animals and livestock but they can also have medicinal benefits. Stock remedies are substances used on animals for a variety of health related purposes, including disease diagnosis and cure as well as improving or maintaining reproduction, growth or general health. Fertilisers are agents used to improve plant growth as well as soil fertility and are broadly categorised into organic and inorganic, the latter being the synthetic and widely applied form in commercial agriculture.

1.4.1.1 Pesticides

The term pesticide refers to an agricultural remedy that includes (amongst others) insecticides, fungicides and herbicides. In terms of the quantity of application these are the most important categories of pesticides used in the country, although other formulations (e.g. rodenticides) are also used in lower quantities. Currently there are in excess of 500 different pesticide active ingredients registered for use in South Africa (PAN, 2013). A pesticide product is essentially a formulation that contains a number of different materials, including active and inert ingredients, as well as contaminants and impurities. The active ingredient is usually the only component of the formulation listed on the pesticide label and is by nature biologically and chemically active against a target pest, be it an insect, weed or fungus. For example glyphosate is the active ingredient in the pesticide product that goes by the trade name of Roundup. For the purposes of this report, the active ingredient is used to identify different pesticides that were identified in sampling programmes and included in subsequent risk assessments.

A review of the literature indicates that many pesticides have been proven to be carcinogenic or endocrine disrupting (FOOTPRINT, 2010). For many pesticides however, there is a large amount of uncertainty as to whether a chemical is definitively a carcinogen or EDC or neither. While a small proportion of pesticides are known to be carcinogenic the majority of pesticides registered for use in South Africa are characterised as non-carcinogenic (Figure 1.1). There are however a relatively large proportion of pesticides that are classified as being possibly carcinogenic (i.e. requiring further research or testing). Only a small proportion of pesticides have no data, indicating that the potential for pesticides to result in carcinogenicity has been relatively well researched. In contrast, the endocrine disrupting (ED) potential of active ingredients of major groups of pesticides is less well understood. A small proportion have been characterised as having either ED potential or not, while the clear majority do not have data. The similar distribution of chemicals that are and are not EDCs implies that a large the large number of chemicals that do not have data could in fact cause ED effects. There is thus a large amount of uncertainty as to the risk posed by current use agrochemicals on carcinogenicity and EDC effects in human and animal health. Considering the importance of the agricultural sector and the number of active ingredients used in the sector, it is therefore important to identify priority compounds used in South Africa that could potentially result in negative effects on human and animal health. This was the main focus of Volume 2 of this research project.

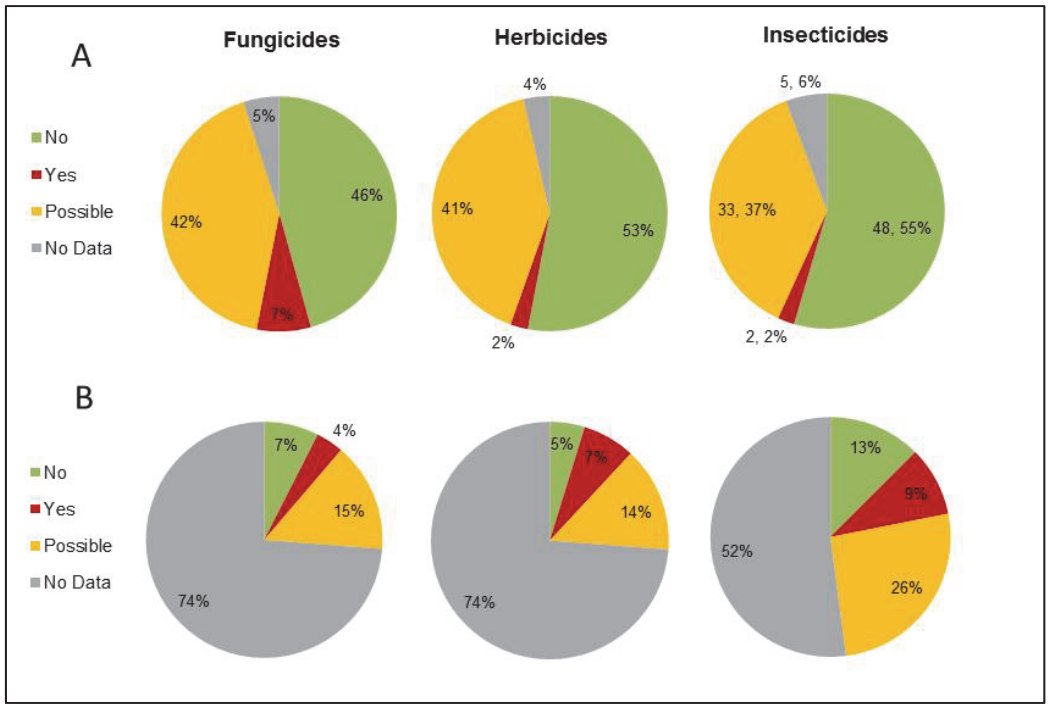

Figure 1.1: Figures illustrating the proportion of active ingredients registered in South Africa for which the potential to cause carcinogenic (A) and endocrine disrupting effects (B) have been established.

1.4.1.2 Fertilisers

Fertilisers are mainly essential growth nutrients based in their formulation, namely nitrogen (N), potassium (K) and phosphate (P). Fertilisers may also contain micronutrient (calcium, sulphur, magnesium) and trace metal (boron, copper, manganese, zinc) elements, depending on the growth requirements. Ammonium (NH_4^+) and nitrate (NO_3^-) fertilisers are the most common forms of N fertilisers; the former being highly volatile and easily converted to the nitrate form. Fertilisers are generally hydrophilic coming in various forms, from liquid to granular forms. Nitrates in particular have been the subject of concern, as due to their high solubility and leaching potential, they easily enter into groundwater, posing a risk to human health. Particularly in rural areas, incidence of methemoglobinemia appears to be the result of high nitrate levels in groundwater drinking resources. Methemoglobinemia, or blue baby syndrome, robs the blood cells of their ability to carry oxygen. More recently, there is growing evidence that nitrates in particular, could illicit ED effects in vertebrates (Guillette and Edwards, 2005).

1.4.2 Environmental Transport

Contamination of water resources by agrochemicals generally occurs via nonpoint source (or diffuse) pollution. Contamination thus occurs over large and disparate spatial and temporal scales and it is difficult to identify specific sources, or areas responsible for pollution. The main route of transport of agrochemicals into non-target surface and groundwater environments is via runoff, leaching and spray drift. Runoff and spray drift typically result in direct contamination of surface waters, whilst

leaching results in contamination of groundwater resources. For all transport pathways, the quantity of the agrochemical used is an important factor that influences the extent to which it is likely to contaminate surface waters. The total quantity used is largely determined by the rate and frequency of application to specific crops for which a pesticide is registered for use. Pesticides vary significantly in their physicochemical properties (i.e. solubility, half-life, K_{OC} etc.) and thus behave differently in terms of their environmental transport and fate. The quantity of use, in combination with various environmental variables and physicochemical properties determine a) which pesticides are most likely to enter water resources and b) the main transport pathway.

In contrast, nitrogen based fertilizers are fairly uniform in their behaviour and are highly soluble, readily entering surface waters and groundwater via runoff and leaching, respectively. In contrast to pesticides, the cost of analysis is low and facilities are readily available for nitrate analysis. Thus water quality monitoring data collected throughout the country through routine monitoring programmes can provide a reasonable picture of the extent of nitrate contamination of surface and groundwater.

1.4.2.1 Runoff

Runoff is the physical movement of excess water along the soil surface. This occurs when the rate of rainfall exceeds the rate of infiltration. Runoff is thus typically associated with heavy thunderstorms, where large volumes of water fall in a relatively short space of time. Runoff may also however occur during irrigation of fields, when the rate of irrigation exceeds the rate of infiltration. Runoff transports both dissolved and soil sorbed chemicals into water resources. The extent to which agrochemicals are associated with the water or sediment phase is primarily dependent on their physicochemical properties. Pesticides with high water solubility and low K_{OC} (soil water partition coefficient) constants will typically be associated with the water phase, whilst less soluble pesticides with high K_{OC} constants are often associated with the sediment phase (Dabrowski *et al.*, 2002). Rainfall events often cover large geographic areas which may contain more than one crop type, and therefore often result in multiple pollutants being mobilised simultaneously over a relatively large area (in both the water and sediment phase) (Dabrowski and Schulz, 2003). Runoff generally results in high peak concentrations for a short duration (a matter of hours) shortly after large rainfall events. Contamination is therefore event driven and pesticide concentrations are transient. This means that routine monitoring programmes are unlikely to effectively characterise pesticide contamination in surface waters as peak levels will often be missed.

Important environmental and physicochemical factors that influence of the mobility of agrochemicals in runoff are listed in Table 1.1. Generally steep sloped agricultural areas, with finely textured, low organic carbon content soils will lead to high potential for surface runoff to occur. As mentioned previously, water solubility and K_{OC} will largely determine the phase with which agrochemicals are associated (i.e. sediment or water). Agrochemicals with long half-lives are also more prone to be associated

with runoff as they are more persistent in the environment and therefore have a greater chance of being present during rainfall events.

In the context of fertilizers, their availability for runoff is dependent on the extent to which they have been taken up by crops. In this context fertilizer management plays a significant role. Nitrates are completely soluble in water and are thus highly vulnerable to runoff and leaching. Excessive nitrate losses can be minimised by ensuring there is as little nitrate in the soil at any one time. This means that nitrates should be applied only when needed (i.e. if there is nitrogen deficiency in the soil) and then only when crops require it (i.e. during the growing season). Excessive nitrate losses can occur when fertilizers are applied in excess of what is required by the crop and when the crop is not using it.

Table 1.1: Summary of surface water contamination potential (via runoff) as influenced by water, pesticide and soil characteristics

	Potential for Surface Water Contamination	
	Low risk	**High risk**
Pesticide characteristics		
Water solubility	Low	High
Soil adsorption (K_{OC})	High	Low
Persistence (Half-life)	Low	High
Soil characteristics		
Texture	Coarse sand	Fine clay
Organic matter	High	Low
Macropores	Few, small	Many, large
Weather characteristics		
Rain/irrigation	Small volumes at infrequent intervals	Large volumes at frequent intervals

1.4.2.2 Leaching

Leaching is the vertical or lateral movement of agrochemicals through soil as opposed to over the soil surface. Leaching is thus largely responsible for contamination of groundwater resources. Again the physicochemical properties of pesticides play a significant role in the potential for a pesticide to move into and through soil. Pesticides with high water solubility and low K_{OC} values are more prone to leaching as they can easily dissolve into water and move with water through the soil (Table 1.2). Those chemicals that have high K_{OC} values have a strong tendency to bind to organic carbon in the soil and are less likely to leach into groundwater. The K_{OC} value is such a good predictor of a chemicals leachability, that Gustafson (1989)

was able to derive a simple scoring system (Groundwater Ubiquity Score Index, or GUS Index), using the K_{OC} and half-life of chemicals as input, that reliably indicates the potential for an agrochemical to leach into groundwater. The GUS score (in combination with other relevant factors) was used as part of the prioritisation process in Volume 2 of this report to rank the environmental mobility of pesticides applied in South Africa.

In contrast to runoff, leaching may result in prolonged contamination of a groundwater resource, particularly for pesticides with relatively high half-lives. With respect to nitrogen fertilizers, their high application rate, coupled with their high water solubility, make them susceptible to leaching and runoff, and high concentrations are found in surface and groundwater.

Table 1.2: Summary of groundwater contamination potential (via leaching) as influenced by water, pesticide and soil characteristics

	Potential Groundwater Contamination	
	Low risk	**High risk**
Pesticide characteristics		
Water solubility	low	high
Soil adsorption (K_{OC})	high	low
Persistence (Half-life)	low	high
Soil characteristics		
Texture	fine clay	coarse sand
Organic matter	high	low
Macropores	few, small	many, large
Depth to groundwater	deep (30 m or more)	shallow (30 m or less)
Water volume		
Rain/irrigation	small volumes at infrequent intervals	large volumes at frequent intervals

1.4.2.3 Spray drift

Spray drift refers to the windblown drift of a pesticide from the point of application (i.e. agricultural field) to a non-target area (e.g. adjacent, non-agricultural land or water body). Due to the direct input and immediate availability of pesticides, spray drift is often regarded as the worst case scenario in terms of pesticide exposure in aquatic environments (Schulz, 2001). Similarly to runoff, spray drift leads to high, yet short-lived, levels of exposure in surface waters (Reichenberger *et al*., 2007). Pesticides transported via spray drift have been detected at large distances away from the area of application. One study detected herbicide up to 150 m off target following application by a tractor boom sprayer (Carlsen *et al*., 2006a). Drift deposits of 2% of

the applied dose were found adjacent to chlorpyrifos vineyard applications (Capri *et al*., 2005). In an Australian study, spray drift of endosulfan insecticide aerially applied to a commercial cotton crop was detected 500 m downwind at ~2% of the field applied rate (Woods *et al.*, 2001). There are a number of factors which contribute to contamination of water resources via spray drift. These include the distance from the point of application to the water resource, wind speed and direction, spraying equipment (e.g. nozzle size) and release height (Hewitt, 2000). In addition to these, it is important to consider physical factors such as the crop canopy and crop distribution, which also affects drift rates (Capri *et al*., 2005).

1.5 ENDOCRINE DISRUPTION

The awareness that chemicals in the environment can have adverse effects on wildlife, and that human health is inextricably linked to environmental health, was highlighted by the publication of Rachel Carson's Silent Spring (Carson, 1962). Some discrepancies are noted in the literature regarding definitions of chemicals and possible adverse effects following exposure. Although definitions regarding chemicals are less variable, those for some classes and types of effects are the subject of some debate, for example, endocrine disruption.

As an example, endocrine disruption may be argued to not necessarily have any physiological significance. The World Health Organisation global assessment on endocrine disruption the International Programme for Chemical Safety definition of an endocrine disruptor is (IPCS, 2002):

> *"an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations."*

The Endocrine Society acknowledged in 2009 that "the understanding of the mechanisms by which endocrine disruptors exert their effects has grown" (Diamanti-Kandarakis, 2009), and now recognizes much broader mechanisms. Thus, in addition to nuclear hormone receptors (e.g. oestrogen and androgen receptors, progesterone receptors, thyroid receptors) effects "via nuclear receptors, non-nuclear steroid hormone receptors (e.g. membrane ERs), nonsteroid receptors (e.g. neurotransmitter receptors), orphan receptors (e.g. aryl hydrocarbon receptor), enzymatic pathways involved in steroid biosynthesis and/or metabolism, and numerous other mechanisms that converge upon endocrine and reproductive systems" are also recognized, with the definition accepted from a physiological perspective as:

> *an endocrine-disrupting substance is a compound, either natural or synthetic, which, through environmental or inappropriate developmental exposures, alters the hormonal and homeostatic systems that enable the organism to communicate with and respond to its environment".*

Although these differences have significant impacts assessing the effects of exposure to agricultural chemicals, for the sake of incorporation this report uses the following concepts:

> *The term "disrupt" may be interpreted in the context of physiology to imply that a biological process has been interrupted to the extent that it has either been stopped, or been prevented from normal continuance.*
>
> *The use of both applications of WHO and US EPA definitions for adverse effects and endocrine disruption.*

It is required to differentiate between different fields of studies when reviewing literature on exposure and effects regarding agricultural chemicals that may be both naturally occurring and synthetic. Toxicological studies investigate the properties of toxicants as well as their biological effects whereas pharmacological studies investigate substances that interact with living organisms through chemical processes, especially by binding to regulatory molecules and activating or inhibiting normal body processes. Both fields of study are applied to prevent, diagnose and treat diseases and disorders. Despite these fields seemingly dealing with two opposite effects, namely one beneficial and the other detrimental, both fields require similar information types and use similar concepts. Furthermore, although pharmacology studies the effects of chemicals (drugs) used at therapeutic doses on organisms whilst toxicology focuses on chemicals that are harmful, both fields overlap significantly, notably with studies on endocrine disruption. Three fundamental commonalities exist in different studies of agricultural chemicals and effects, namely (Katzung, 1998; US EPA, 1997; 1998):

- Sources
- Pathways
- Receptors

Although not dealt with in great detail in this chapter it should be noted that relevant issues in linking exposure to effects include:

- Time of exposure:
    - If exposure occurs during the developmental stage of the endocrine system the consequent effects may result in a permanent change or loss of function or sensitivity.
    - If exposure occurs in adulthood normal homeostatic mechanisms may be sufficient to compensate for the effects with no significant or clinical adverse effects observable.
- Context of exposure:
    - The same degree of exposure may have different effects in individuals with different histories or different seasonal exposures.
- Due to feedback mechanisms and numerous links (typical of endocrine disruption) exposure may result in unpredictable effects both in terms of the target tissues and type of adverse effect.

- Due to the complexities between the large number of potential chemicals an organism can be exposed the outcomes of concurrent exposure to multiple chemicals are very difficult to predict. Effects may range from additive, supra-additive (synergistic) to infra-additive (antagonistic). Potentiation may also occur where effects are only noted when specific combinations of chemicals are present in the exposure mixture. Failing evidence to the contrary it is normally assumed the effects are synergistic.

1.6 RISK ASSESSMENT

Assessments also investigate hazards, which are different from risks as they relate to descriptive terms that characterize the intrinsic capability of a chemical to cause harm. A hazard is in effect a source of risk and with regard to a chemical influenced by a variety of factors such as persistence, mobility, toxicity, and carcinogenicity. To understand the environmental health implications of agricultural chemicals a health risk assessment process will be adopted in this study. The health risk assessment process comprises four distinct, but interacting phases, namely:

Hazard Identification establishes whether exposure to a chemical or microbiological agent can cause harm. The process encompasses the description of the properties of the hazardous agent, and the identification of both acute and chronic health effects.

Dose-response Assessment characterises the relationship between the dose of a hazard agent (i.e. the amount of pollutant taken into the body through inhalation, ingestion and dermal contact) and incidence of an adverse effect in the exposed pollution.

Exposure Assessment establishes the intensity, frequency and duration of human contact with a contaminant. To determine exposure, it is necessary to combine an estimation of environmental concentrations of the hazards with demographic or behavioural descriptions of the exposed population.

Risk Characterisation provides an indication of the incidence of the health effect under the conditions of exposure described in the exposure assessment and the identified dose-response relationship. The risk estimates are presented according to the type of health effect that might arise from the chemical contaminant. Chemicals that are hazardous may be either carcinogenic or toxic (or both).

Exposure to pesticides can take place via a number of routes and media. This includes contamination of food, air, soil, or water. These different media may enter the body through ingestion, inhalation or dermal absorption. Figure 1.2 presents a schematic depiction of the many routes pesticides might take to enter a human. Water is one of these routes and may become contaminated through run off after application to land. In addition to direct application of chemicals to crops, they may become contaminated if irrigated with water containing pesticides. Pesticides in water can settle to the sediment, causing fish to become contaminated bio-accumulating

the pesticide. A human health risk assessment process needs to consider the different pathways and media to predict the possible health effects that may occur.

In many instances there is a lack of appropriate data for all risk assessment steps which sets significant challenges to performing assessments for exposure and effects. It is important to note that not all investigations need to complete this process as the objective may not always be to perform a risk assessment. Each of these steps in the risk assessment process contribute to our understanding of exposure to agricultural chemicals and, in conjunction with the precautionary principle and expert opinion based on accepted scientific evidence, may be used to formulate corrective measures, guidelines, and/or actions that relate in principle to risk management.

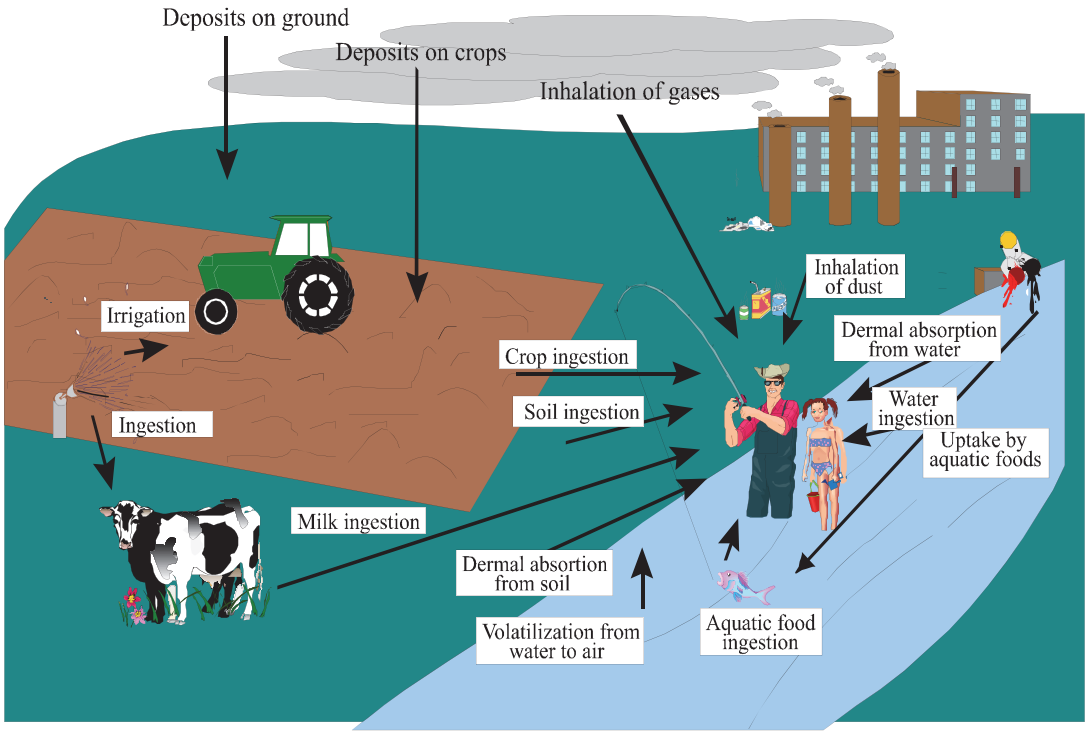

Figure 1.2: Exposure pathways of pesticides

1.6.1 Risk Assessment of EDCs

It is difficult to establish cause and effect relationships for human exposure of endocrine disrupting chemicals (EDCs) and incidence disease. We encounter a broad range of environmental exposures throughout our lives and frequently to a mixture of chemicals at any one time. This makes it difficult to know which chemicals at what dosages are harmful. Another challenge is that endocrine disruptors have trans-generational capabilities. This means they could elicit effects up to a second or third generation after exposure. Also, health effects due to prenatal exposure may not present themselves until later in life. It is generally believed that the earliest stages of life are the most sensitive to endocrine disruption.

Endocrine disruption in brief involves the interference with the normal functioning of the endocrine system in three possible ways:

- By mimicking the action of a naturally-produced hormone, such as oestrogen or testosterone, and thereby setting off similar chemical reactions in the body;
- By blocking the receptors in cells receiving the hormones, thereby preventing the action of normal hormones; or
- By affecting the synthesis, transport, metabolism, and excretion of hormones, thus altering the concentrations of natural hormones.

The endocrine system regulates processes as diverse as blood pressure, smooth muscle contraction, fluid balance and bone-resorption (IPCS, 2002). For many of the systems the setup is programmed during foetal development and an abnormal environment during this critical stage can result in permanent mis-programming (IPCS, 2002). At least four major categories of adverse biological effects may be linked to exposure to endocrine disruptors, namely:

- Cancer,
- Reproductive and developmental alterations,
- Neurological effects and
- Immunological effects.

Endocrine systems that may be involved include the thyroid, adrenal, pituitary, and gonadal system. This includes cognitive effects, which have been observed in animals and humans (Schantz and Widholm, 2001).

A concern regarding the bioaccumulation of certain chemicals has the potential for embryos and infants to be exposed at critical stages in their development through the womb or through their mothers' breast milk. The reproductive system and embryo are the two bodily systems that seem particularly vulnerable (ACS, 1998). Based on this, the majority of the studies have focused on reproductive health effects. A number of adverse reproductive health effects have been observed in which endocrine disrupters could play a role.

There is no standardised method or guideline to assess human health risks associated with endocrine disrupting chemicals (WHO, 2004). A previous study (Genthe *et al.*, 2009) suggested a framework for endocrine disrupting chemical guidelines for drinking water for South Africa based on a tiered approach. First level screening tests for reproductive endocrine disrupting capability was recommended rather than testing specific or individual chemical concentrations. Screening using a battery of *in vitro* and *in vivo* tests quantitatively expressing the results of oestrogen activity of a water sample containing a mixture of chemicals in terms of their relative potency was recommended. An approach similar to toxic equivalency factors was recommended specifically for oestrogen activity and their activity in water expressed in terms of equivalency factors. A value above which a more detailed assessment is recommended is the "trigger value". If endocrine disruption is detected at concentrations higher than the specified "trigger value", then a more detailed assessment is recommended to identify the chemicals responsible.

1.6.2 Bioassays to detect endocrine disrupting effects.

1.6.2.1 Bioassays to detect endocrine disrupting effects by compounds

The Organisation for Economic Co-operation and Development (OECD) as well as the Environmental Protection Agency of the Unites States of America (US EPA) are developing and validating both *in vitro* as well as *in vivo* assays to determine whether a substance has a possible endocrine disrupting effect. They are focussing on three hormone systems: oestrogen, androgen and thyroid. Their tier 1 tests include a screening battery while the tier 2 tests intend to confirm, characterize, and quantify those effects (US EPA, 2010).

The tier 1 screening assays involve the amphibian metamorphosis assay, receptor binding *in vitro* assay, aromatase assay, the fish screen assay, the Hershberger assay, pubertal female and male assays, steroidogenesis *in vitro* assay, uterotrophic and the 15-day adult intact male assay.

In the amphibian metamorphosis assay tadpoles are exposed to chemicals to determine if these chemicals affect the thyroid during metamorphosis. Developmental effects are a positive sign of affected thyroid (US EPA, 2010). A variety of endpoints can be measured using this assay: mortality, malformations, body weight, percentage of metamorphosed froglets at the end of the exposure period, percentage of tadpoles in different developmental stages and developmental stage-dependent awarded penalty points (Gutleb *et al*., 2007).

One of the mechanisms through which chemicals can affect the endocrine system is by binding to hormone receptors and act as the natural hormone would or block access of the hormone to the site and in this manner inhibit hormone controlled activity. Of the variety of receptor based assays available in literature, the US EPA are validating the androgen receptor binding assay that uses rat prostate cytosol and the oestrogen binding assay that uses rat uterine cytosol as a source of receptors. The aromatase *in vitro* assay investigates that section of the steroidogenic pathway that detects compounds capable of inhibiting aromatase activity. Aromatase is an enzyme complex that converts androgens into oestrogens, oestradiol and estrone. The fish screen detects both oestrogenic and androgenic effects. Endpoints such as survival, reproductive behaviour, secondary sex characteristics, histopathology and fecundity end points are evaluated in fish exposed to compounds of interest (US EPA, 2010).

The steroidogenesis assay is another *in vitro* assay that utilises the H295R human adrenocortical carcinoma cell line. This cell line can detect compounds which influence those enzymes responsible for steroid synthesis. It measures the expression of steroid hormones in exposed cells and compares it to unexposed cells (Hecker *et al*., 2007; US EPA, 2010).

The Hershberger assay is an *in vivo* assay where castrated or immature male rats are exposed to the compounds of interest and the accessory sex gland weights as well as other androgen-dependent tissues are measured. Other *in vivo* assays involve the use of pubertal male and females rats to screen for androgenic/oestrogenic and thyroid activity during sexual maturation. Abnormalities

associated with sex organs and puberty markers, as well as thyroid tissue are investigated (US EPA, 2010). The uterotrophic assay is also an *in vivo* assay and involves the use of female rats to screen for oestrogenic effects. Uterine weight changes are measured in ovariectomised or immature female rats. The 15-day adult intact male *in vivo* assay screens for abnormalities associated with primary and secondary sex organs, systemic hormone concentrations, and thyroid using male rats (US EPA, 2010). The tier 2 testing assays are also under consideration and include test animals such as frogs, birds, fish, mammals as well as invertebrates. For complete details on the status of the assays considered by the US EPA and the OECD visit the US EPA's web site.

Burger (2005) listed six South African institutions which between them had a number of bioassays available. These institutions included universities such as the University of Stellenbosch, Pretoria and the North-West University, the CSIR and two state departments, the then Department of Water Affairs and Forestry and the Department of Trade and Industry. The tests available between the institutions varied between the reporter gene bioassays based on mammalian and human cancer cells as well as the recombinant yeast, vitellogenin, catfish vitellogenin assay and thyroid function *Xenopus* assay. Since this publication, the Water Research Commission had funded research on specifically endocrine disrupters in the aquatic environment, with special reference to those with oestrogen activity. As a result of this research a toolbox of bioassays to detect oestrogen activity has been compiled (De Jager *et al*., 2010) and is in the process of being published as a laboratory manual.

1.6.2.2 Bioassays to detect endocrine disrupting effects in water

The oestrogen activity detection bioassays for the aquatic environment in South Africa were selected after studying a 2008 publication by the Global Water Research Coalition (GWRC), "Tools to detect oestrogenic activity in environmental waters" (Leusch, 2008). The Global Water Research Coalition (GWRC) was formed in 2002 by the water research institutions of amongst others, the USA, Australia, Switzerland, Netherlands, France and South Africa. This publication was a culmination of a research project into selecting the most appropriate *in vivo* bioassays to determine oestrogen activity in the natural environment. An extensive literature survey resulted in the identification of 24 bioassays suitable to measure oestrogenic activity in environmental waters. Five of these assays were selected for validation. The criteria to which a bioassay is measured included global applicability, reliability, robustness, maturity and potential for high-throughput screening. The bioassays tested during this project were the yeast oestrogen screen (YES), the ER-CALUX, the MELN, theT47D-KBluc and the MCF7 cell proliferation (E-Screen) assays. In Leusch and co-workers (2010) these latter named assays were compared regarding their sensitivities and repeatability in detecting oestrogen activity in environmental waters. The ER-CALUX and E-Screen bioassays correlated well with chemical analysis and were predictable. The T47D-KBluc assay was promising too, but the YES assay was less sensitive than the other assays by an order of magnitude. The MELN assay was less predictable.

Yeast Oestrogen Screen (YES)

Yeast cells have been stably transfected with the gene for human ERα and a plasmid containing an oestrogen-response-element (ERE)-linked lac-Z gene. This gene is also expressed when a compound binds to the receptor and produces the enzyme β-galactosidase. The substrate of this enzyme is a yellow chromogenic compound, chlorophenol red- β-D-galactopyranoside. When the substrate is given to the cells, the galactosidase enzyme transforms its yellow substrate into a red compound. The concentration of the latter is determined in a spectrophotometer at 540 nm. The ability of an environmental extract to cause the expression of β-galactosidase is expressed in relation to the ability of a reference compound, 17β-estradiol (E_2) with a known concentration and is called estradiol equivalents (EEq) (De Boever *et al*., 2001).

Although this assay is the most commonly used one, cytotoxicity might be a problem in highly concentrated water samples. The yeast cell wall may prevent the transport of compounds to the inside, increasing the risk of false negatives. This assay was selected to be included in the SA Toolbox (De Jager *et al*., 2010).

ER-CALUX

Oestrogen reporter chemical-activated luciferase gene expression assay (ER-CALUX) uses the T47D human breast cancer cells stably transfected with an ERE luc plasmid. The principle this assay is based on is the same as for the yeast screen described in the previous paragraph. The enzyme that is expressed in the presence of a ligand agonist is luciferase and its substrate is luciferin. In this assay, however, light is emitted when the substrate is given to the cells. This luminescence is determined by an instrument as relative light units (RLUs). The RLUs are directly proportional to the amount of ligand (oestrogen-like compounds) in the environmental extract. Again, the luminescence elicited by the sample extract is expressed as a percentage of the luminescence elicited by the reference compound (E_2) and EEq is calculated (Legler *et al*., 1999).

ER-CALUX is, however a patent of BioDetection Systems in the Netherlands and because of annual licence fees an expensive assay. For this reason it was not included in the SA Toolbox.

MELN

The principle of this assay is very similar to the previous one, except that a different cell line was stably transfected. MCF-7 human breast cancer cells have been transfected with the luciferase gene (luc) driven by an ERE in front of the β-globin promoter. These cells have both the oestrogen receptors, α and β endogenously. The assay is performed along the same regime as described previously for the ER-CALUX (Fenet *et al*., 2003).

This assay was not included in the SA Toolbox.

T47D-KBluc

This assay is very much the same as the ER-CALUX. The same cell line was used to genetically modify T47D cells were stably transfected with a triplet ERE-promoterluciferase reporter gene construct (Wilson *et al*., 2004). This cell line is more affordable and readily available as CRL-2865 from American Type Culture Collection (ATCC). This assay was included in the SA Toolbox.

E-screen

This assay also uses the MCF-7 human breast cancer cells. These cells are oestrogen-dependent for growth and it is this endpoint that is used to determine if an environmental sample contains oestrogen agonist capabilities. After 5 days of exposure to the environmental sample, the number of live cells are determined and compared to the original number (Körner *et al*., 1999). The standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay is used to determine cell proliferation.

This assay can also lead to false positives as there are compounds, other than oestrogens, capable of inducing cell growths, such as mitogens, cytokines, growth factors, nutrients and other hormones. This assay was included in the SA Toolbox.

Fish vitellogenin assay

The only other assay that was included in the SA Toolbox is an *in vivo* assay based on the vitellogenin levels of juvenile Mozambique tilapia (*Oreochromis mossambicus*) after exposure to environmental extracts. This assay was not assessed in the GWRC publication (Leusch, 2008) that was referred to at the start of section 6.3.2. This assay measures two endpoints: (1) the quantitative expression of vitellogenin (VTG) mRNA in the liver and the quantification of circulating VTG in blood. VTG concentration is done using ELISA, antigens (VTG complex) or antibodies (anti-VTG antibodies). The VTG lipoprotein complex of the Mozambique tilapia is detected with a specific anti-VTG antibody (commercially available antiseabream VTG) in a competitive ELISA approach. For the details of the assay as well as the additional endpoints that can be measured, i.e. the histological work, refer to De Jager *et al.,* (2010).

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CHAPTER 2: STUDY AREAS AND SAMPLING SITES

by

M. Thwala, J.M. Dabrowski and J.M. Shadung

CSIR, Natural Resources and Environment, PO Box 395, Pretoria, 0001

2.1 SITE SELECTION

The process of study area selection was on one hand, based on a list of suggested agricultural locations during the first project workshop held in May 2010. Secondly, the study area selection was informed by requirements as given by the project team members undertaking various study components. Table 2.1 provides a list of suggested study locations that were recommended during the workshop as well as a list of basic requirements for a study location. Briefly; an area with high crop production activity as a source of agricultural chemicals to the environment was a prerequisite. Additionally, human settlements in close proximity with domestic crop and livestock farming activities utilising potentially contaminated water resources with agricultural chemicals were also needed to account for various exposure routes. Emphasis was also placed towards selecting an area with as little impacts as possible from other significant activities that may be a source of EDCs (e.g. industry and mining).

Table 2.1: A list of recommended study locations as well as the basic study area characteristic requirements (selected study areas are highlighted in bold).

Suggested locations for a study area	Study area requirements
Overberg region (Western Cape) Burgershall (Mpumalanga) **Komatipoort (Mpumalanga)** **Bothaville (Free State)** **Letsitele (Limpopo)** Burgersfort (Limpopo) Lower Orange River region (Northern Cape)	Domestic livestock availability Water use by communal/domestic gardens Domestic water use Potential spray drift to human settlements Commercial crop production

The suggested study areas in Free State, Mpumalanga and Limpopo provinces were visited over three days (6-8 October 2010) to closely assess the sites and get more information as to their suitability for the requirements of this study. These areas were preferred because of logistical advantages considering that most project activities would be undertaken mainly from Gauteng province.

While the criteria listed in Table 2.1 describe a relatively high probability of exposure to chemicals (and thus a relevant study site), it was in fact not necessary that these criteria were always met. For example, since no human pathology investigations were undertaken; the source of domestic water supply, either from a formal supply or direct from the resource is not important, as exposure towards humans was assessed using theoretical exposure parameters typically used in human risk assessment studies (see Table A1 in the Appendix). However should high risk be

identified it would be important to then identify areas where the criteria in Table 2.1 do exist, as these would be highly vulnerable communities.

Three of the recommended study areas were selected for further monitoring and assessment studies in this project. The Letsitele catchment was considered a suitable study area as it met most of the criteria. The Lestitele area is a well-known commercial agricultural area producing a wide variety of tropical and citrus fruits. Human settlements with domestic livestock and communal gardens exist in close proximity to commercial agriculture with high potential for exposure via spray drift. Informal conversations with residents in Mogoboya and Lhujwana confirmed that municipal water supply was often interrupted, and water for domestic uses (including drinking) was frequently collected directly from the river (confirmed during field observations). The domination of the Lomati catchment by a single crop, sugar cane, was identified as a distinct advantage as it avoided the complication of assessing the impact of multiple crops and potentially multiple pesticides. A water purification works was identified in the town of Langeloop which extracted water directly from the Lomati River and distributed it to residential homes for drinking water purposes. This served as an ideal site for assessing risks associated with a domestic drinking water supply. The vicinity around Viljoenskroon and Kroonstad (close to Bothaville) was also selected due to the dominance of the area by maize. As maize is the most widely produced crop in South Africa it accounts for a high proportion of pesticide use and should therefore be regarded as a priority when assessing risks associated with agricultural chemical use. The location of the three selected study areas is shown in Figure 2.1.

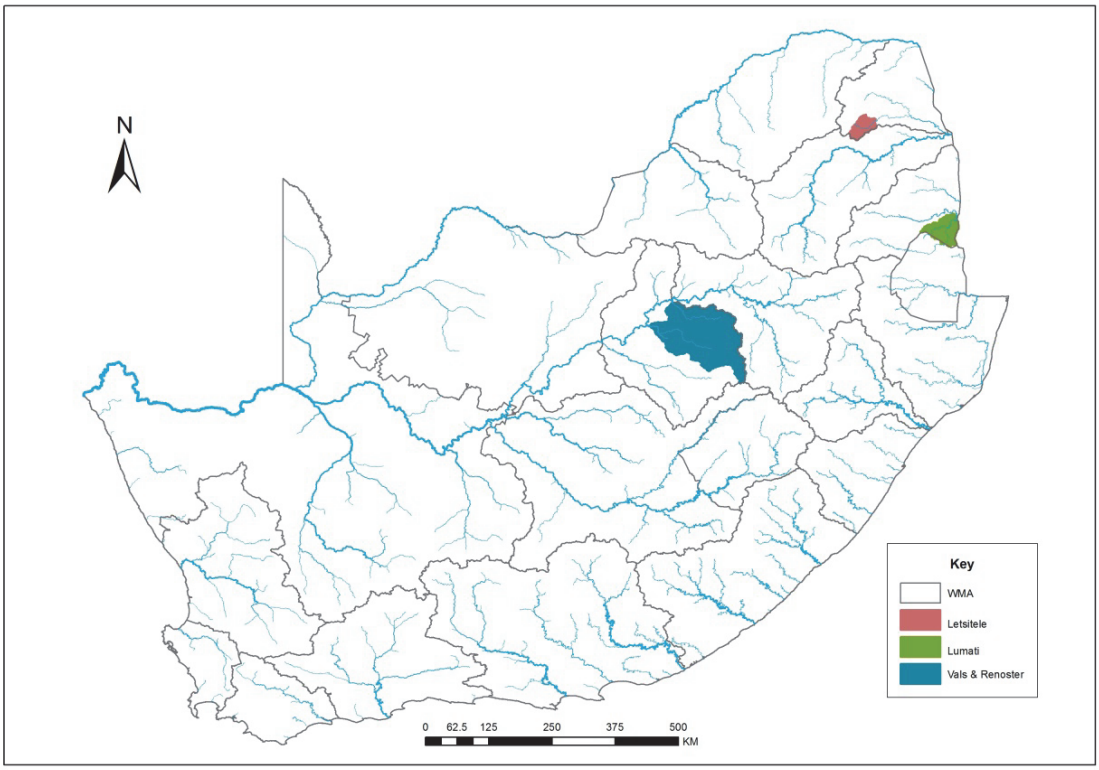

Figure 2.1: Map showing the geographical location of the three study areas in South Africa.

2.2 LETSITELE CATCHMENT

The Lestitele River drains from the Wolkberg mountains and flows in a north easterly direction towards the town of Letsitele, where, shortly after it joins the Letaba River (Figure 2.2). The upper reaches of the catchment are comprised mainly of commercial forestry. Further downstream the river passes through a well-known intensive commercial agricultural area, where avocados, citrus and mangos are most commonly grown. Numerous human settlements, with domestic livestock and communal gardens exist in close proximity to commercial agriculture with high potential for spray drift towards human settlements. The towns of Khujwana and Mogoboya are located adjacent to the Letsitele River in close proximity to fruit orchards. The upper sections of the catchment are characterised by relatively steep slopes (Figure 2.3), resulting in relatively high runoff potential of pesticides. The main soil type is a sandy clay loam (Figure 2.4), which has relatively high percentage of sand and is thus susceptible to leaching of agrochemicals.

Originally, 10 sites were selected within the study area, with the objective being to gain a broad overview of contamination in multiple water resources and sources. Six sites were selected within the main stem of the Letsitele River (LT3, -7 and -9), its tributaries (LT1 and -10) and in the Letaba River (LT8). One site was selected within a dam (Murle Brook dam – LT2). Sites within the Letsitele River were regarded as potential high use sites (for domestic and livestock purposes). The tributary sites were selected for the purposes of identifying relevant agrochemicals used in the area and for the purposes of identifying important sources of chemicals within the catchment area. Three sites were selected at drinking water taps. One was located at a primary school (LT4) and two at community drinking water taps in the Khujwana settlement (LT5 and LT6). These sites were selected for the purposes of identifying pesticides and EDC activity directly in the main drinking water supply. Full details of the sites can be viewed in Table 2.2, while the sampling programme adopted is highlighted in Table 2.3.

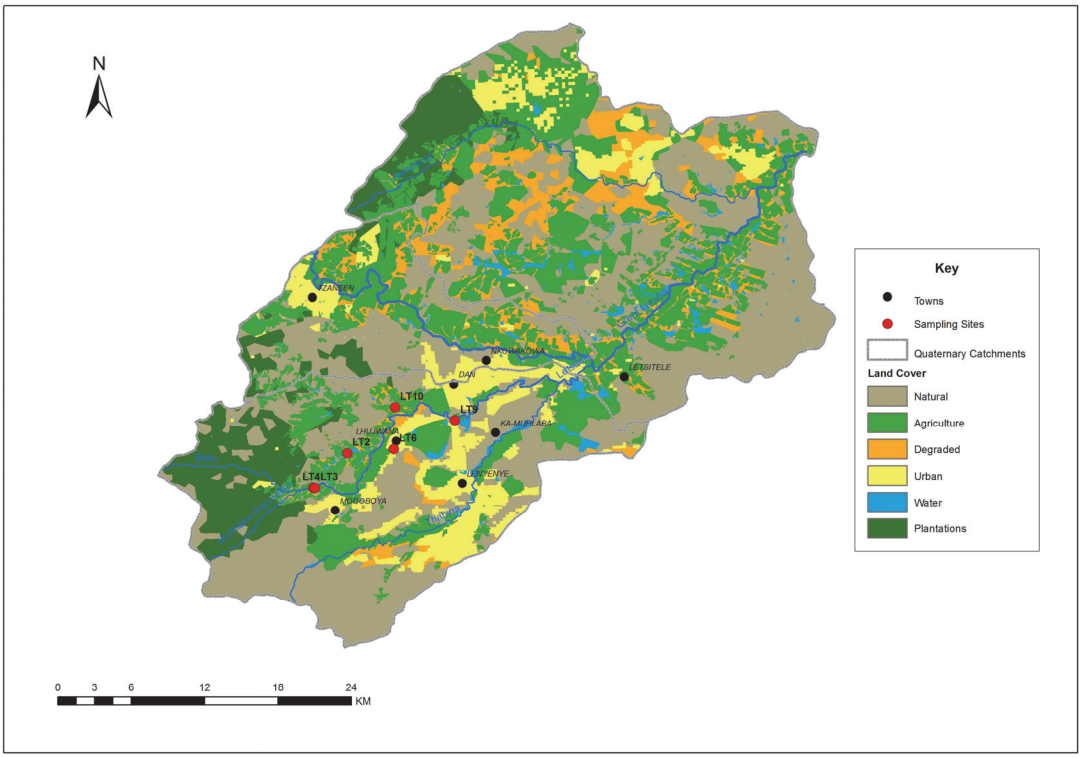

Figure 2.2: Land cover of the Letsitele catchment area.

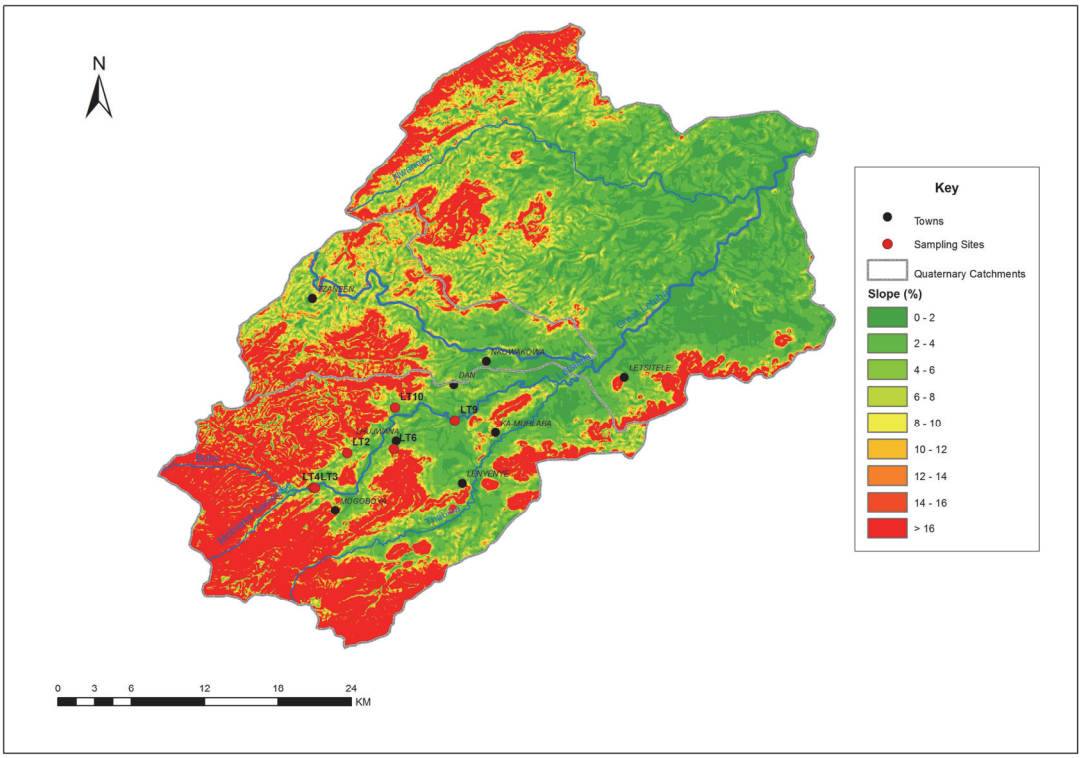

Figure 2.3: Slope characteristics of the Letsitele study area.

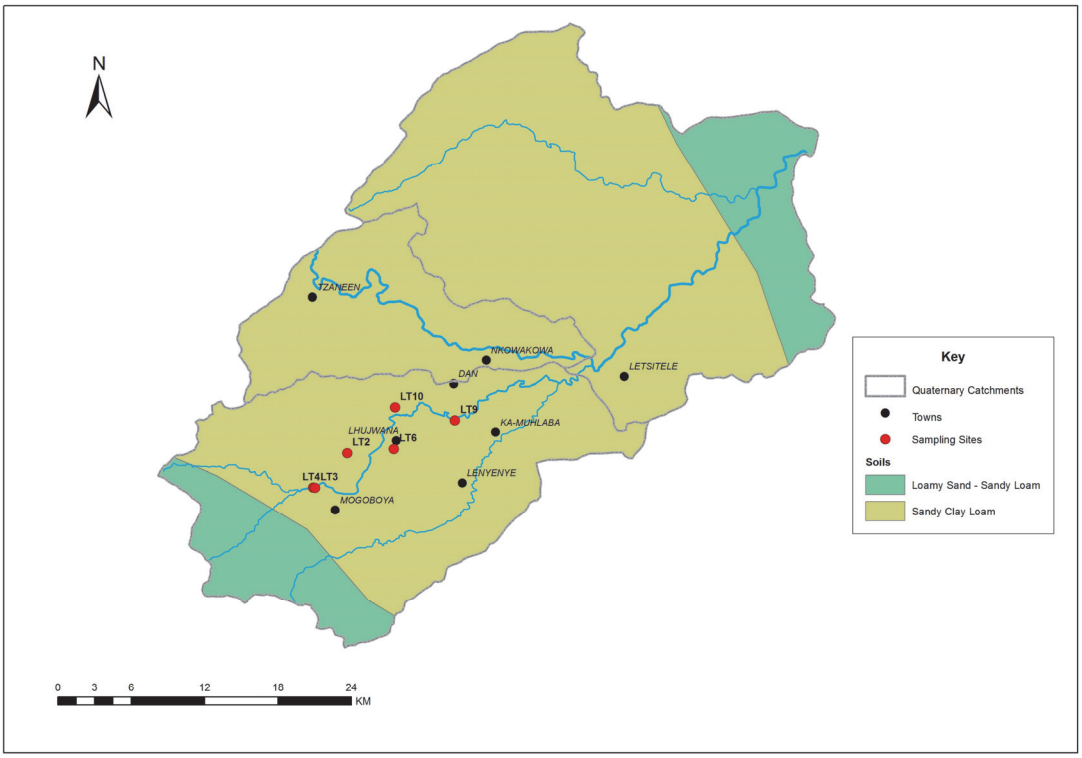

Figure 2.4: Soil texture characteristics of the Letsitele study area.

2.3 SITE DESCRIPTIONS

Table 2.2: Location of selected study sites in the Letsitele River catchment.

Site	GPS co-ordinates	Brief site description
LT1	-23.942679° 30.191409°	Tributary of the Letsitele River at the intersection with the Old Coach Rd.
LT2	-23.950230° 30.188589°	Murle Brook River (in the dam)
LT3	-23.974673° 30.165144°	Upper most site in the Letsitele River
LT4	-23.974653° 30.164833°	Tap at a primary school (right next to Site 3)
LT5		Water collected from a household in the community
LT6	-23.978001° 30.206015°	Tap in the community
LT7	-23.940713° 30.215054°	Middle site in the Letsitele River
LT8	-23.865628° 30.391796°	Site in the Letaba River
LT9	-23.924655° 30.267180°	Lower most site in the Letsitele River
LT10	-23.915846° 30.222248°	Tributary of the Letsitele River, next to a fruit packing shed

2.3.1 Sampling Programme

Table 2.3: Summary of samples collected at sites in the Letsitele catchment ('X' indicates a sample was collected).

Site	Pesticide Analysis				EDC Bioassays		Inorganic Chemistry
	Screening	Quantitative	Water	Sediment	Water	Sediment	
July 2011							
LT1	X		X	X	X	X	X
LT2	X		X	X	X	X	X
LT3	X		X	X	X	X	X
LT4	X		X		X		X
LT5	X		X		X		X
LT6	X		X		X		X
LT7	X		X	X	X	X	X
LT8	X		X	X	X	X	X
LT9	X		X	X	X	X	X
LT10	X		X	X	X	X	X
November 2011							
LT1	X		X	X	X	X	X
LT2	X		X	X	X	X	X
LT3	X	X	X	X	X	X	X
LT4	X	X	X		X		X
LT5	X		X		X		X
LT6	X	X	X		X		X
LT7	X	X	X	X	X	X	X
LT8	X		X	X	X	X	X
LT9	X	X	X	X	X	X	X
LT10	X		X	X	X	X	X
August & November 2012, February 2013							
LT3		X	X	X	X	X	X
LT4		X	X		X		X
LT6		X	X		X		X
LT9		X	X	X	X	X	X
LT10	X	X	X	X	X	X	X

In addition three borehole samples (BM, SL and FM) were collected during August 2013. These samples were subjected to the full suite of analyses listed in Table 2.3. All samples were collected from point of use taps located on three separate fruit farms in the catchment.

2.4 VALS AND RENOSTER CATCHMENTS

The Renoster and Vals catchment areas consists of intensive maize (and to a lesser extent sorghum and sunflower) production and were identified as a potential study area during a site selection workshop. Furthermore, maize production was identified as a high priority crop during the chemical prioritisation process (Table 3.1 and Volume 2 of this report). The catchment is dominated by agricultural land use Figure 2.5), is relatively flat (particularly in the lower reaches - Figure 2.6) and has a high proportion of sandy soils (Figure 2.7) with a relatively high associated leaching potential.

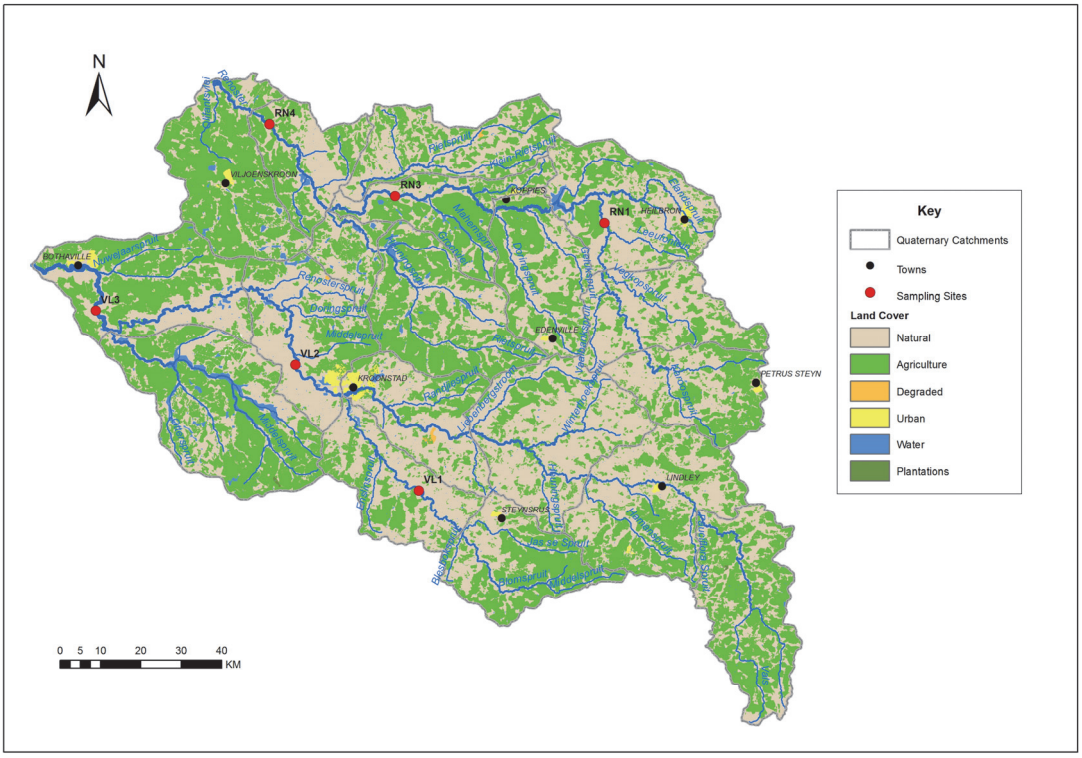

Figure 2.5: Land cover of the Renoster and Vals River catchments

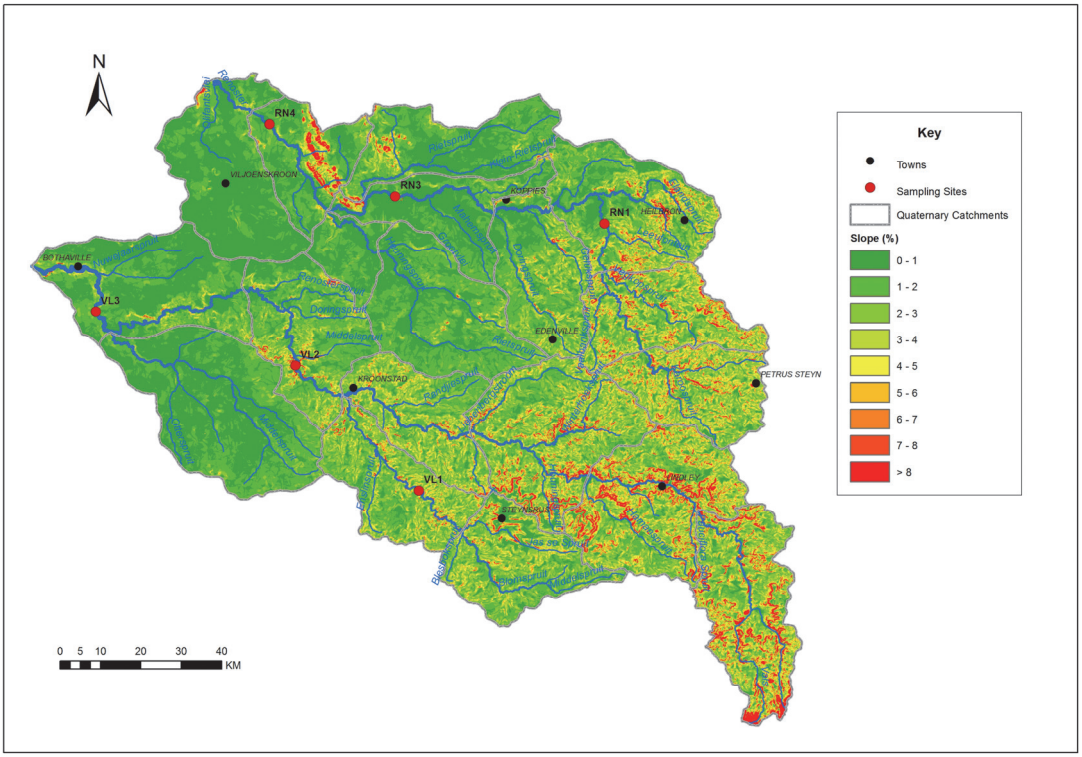

Figure 2.6: Slope characteristics of the Renoster and Vals River catchments.

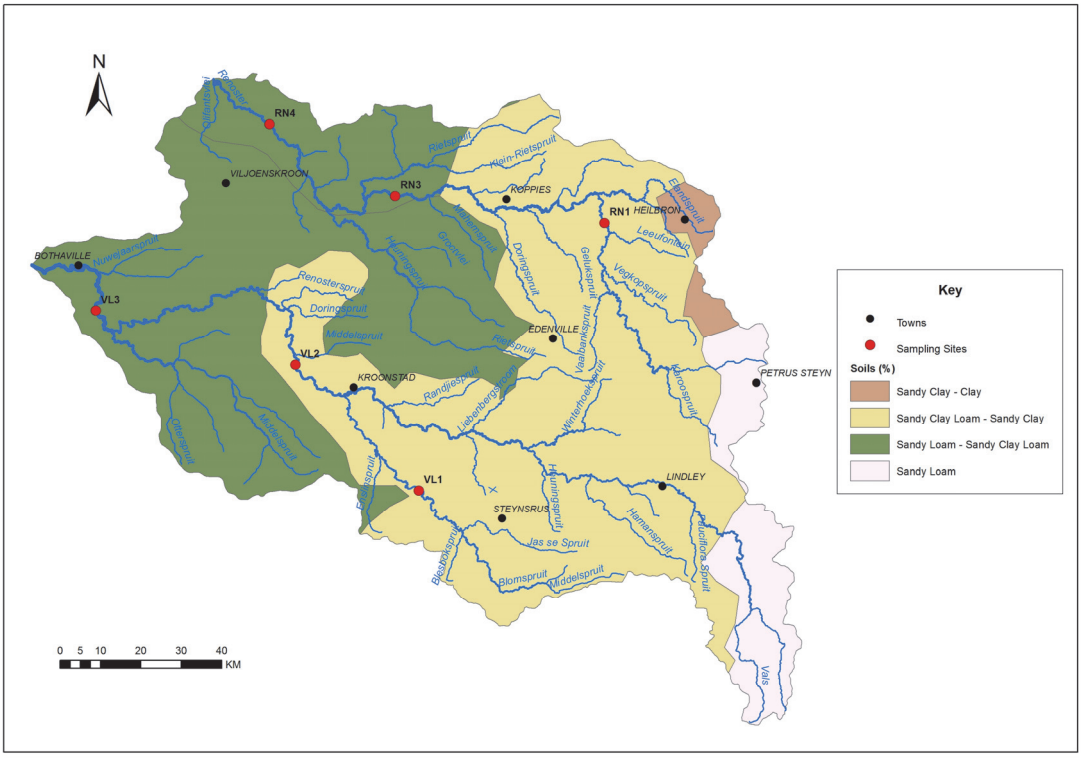

Figure 2.7: Soil texture characteristics of the Renoster and Vals River catchments.

The Vals River flows from upstream of Kroonstad in a westerly direction past Khotsong and Bothaville before entering the Vaal River. The town of Kroonstad is relatively large and is located midway along the river in between VL3 and VL2 and is characterised by a number of industries as well as point source pollution points (e.g. sewage outfalls). The Renoster River also originates in an intensive agricultural area and flows northwards, meeting the Vaal River upstream of its confluence with the Vals River. Relatively few large towns are located along the length of the river.

2.4.1 Site Descriptions

Table 2.4: Location of selected study sites in the Renoster and Vals River catchments.

Site	GPS co-ordinates	Brief site description
RN1	-27.293820° 27.789787°	Upstream of Koppies dam on the Renoster River
RN3	-27.233362° 27.324581°	Downstream of Koppies dam and town, on the R721 crossing the Renoster River
RN4	-27.071720° 27.044392°	Renoster Rive, approximately 16 km upstream of confluence with Vaal River
VL1	-27.890709° 27.376506°	Blom River (tributary of the Vals River) upstream of the town of Kroonstad
VL2	-27.610978° 27.102011°	Vals River, downstream of the town of Kroonstad
VL3	-27.490141° 26.656111°	Vals River, just upstream of the town of Bothaville

2.4.2 Sampling Programme

Table 2.5: Summary of samples collected at six sites in the Renoster and Vals catchments ('X' indicates a sample was collected).

Site	Pesticide Analysis				EDC Bioassays		Inorganic Chemistry
	Screening	Quantitative	Water	Sediment	Water	Sediment	
			February 2012				
RN1							
RN3							
RN4	X		X				
VL1							
VL2							
VL3	X		X				
			July & October 2012, January & April 2013				
RN1		X	X	X	X	X	X
RN3		X	X	X	X	X	X
RN4	X	X	X	X	X	X	X
VL1		X	X	X	X	X	X
VL2		X	X	X	X	X	X
VL3	X	X	X	X	X	X	X

In addition three borehole samples (VJK, KOP and KOS) were collected during August 2013. These samples were subjected to the full suite of analyses listed in Table 2.5. VJK was collected from a borehole tap located in a residential household in the town of Viljoenskroon (Renoster catchment). KOP was selected from a farm in the vicinity of the town of Koppies (Renoster catchment). KSD was collected from a farm in the vicinity of Kroonstad. All boreholes were utilised for domestic and drinking water purposes.

2.5 LOMATI CATCHMENT

The Lomati study area is located in the eastern part of Mpumalanga, north of Swaziland and of Mozambique. Agriculture is largely sugar cane, with a smaller proportion of citrus (Figure 2.8). The Lomati River originates in Swaziland, flows through Driekoppies Dam and finally into the Komati River. Four sampling sites were located within this catchment (Table 2.6). At NK3, additional samples were collected from a tap at the Water Treatment Works in Langeloop. This facility draws water directly from the Lomati River, treats the water and distributes it for domestic use within the town. Additional sites were selected at the end of the Ngwezi River (NK4) and the Mzinti River (NK5). NK4 was downstream of relatively intensive agricultural area. In contrast, NK5 was relatively less impacted by agricultural activity and is immediately below the northern most boundary of the Mahushe Shongwe Provincial Reserve. Agricultural land is generally situated on low to medium gradient slopes (Figure 2.9). Soils in the catchment are generally relatively sandy (Figure 2.10).

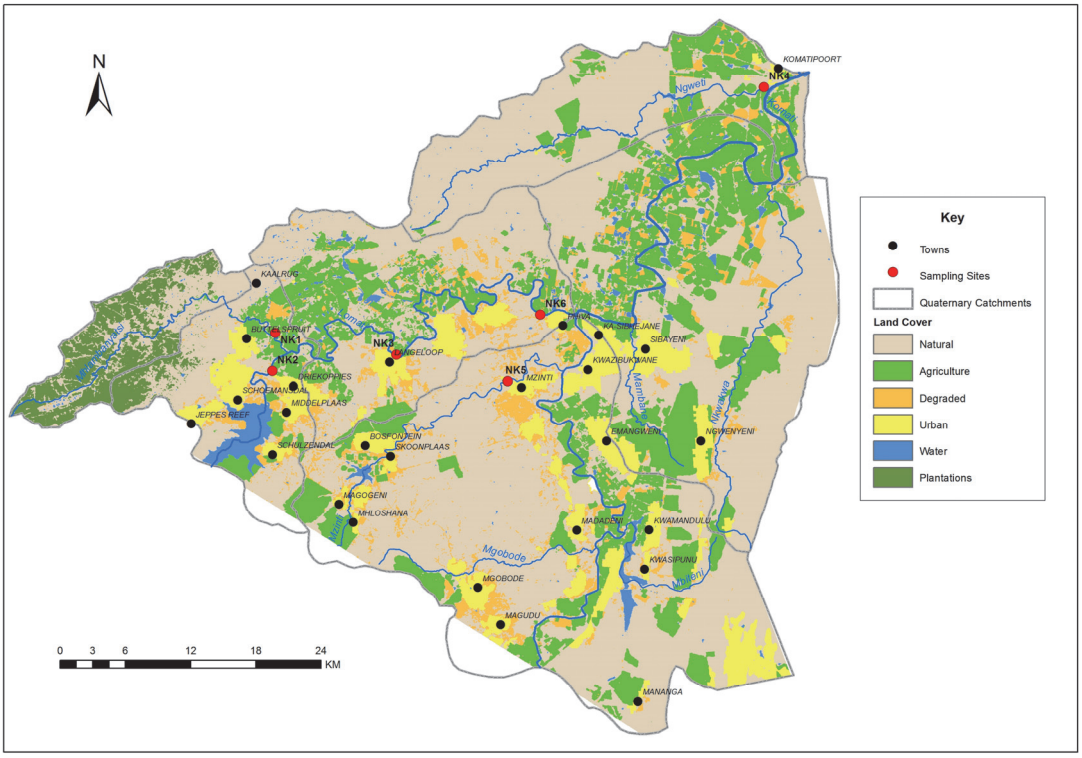

Figure 2.8: Land cover of the Lomati, Mzinti and Ngwezi River catchments.

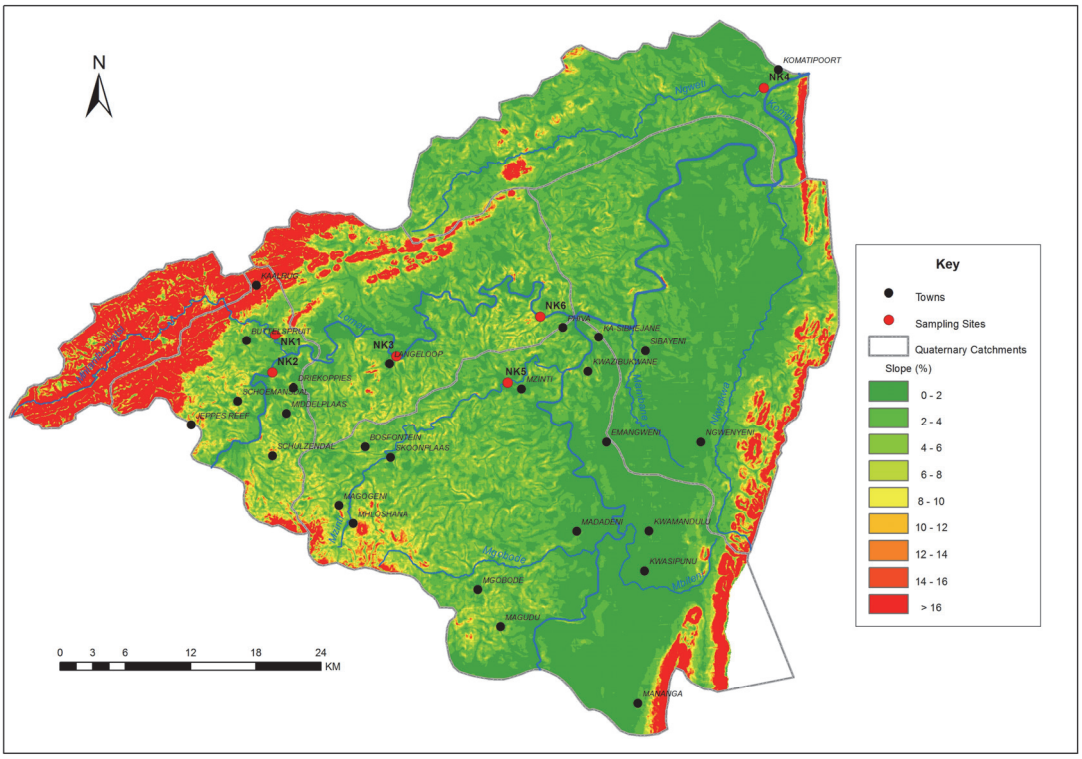

Figure 2.9: Slope characteristics of the Lomati, Mzinti and Ngwezi River catchments.

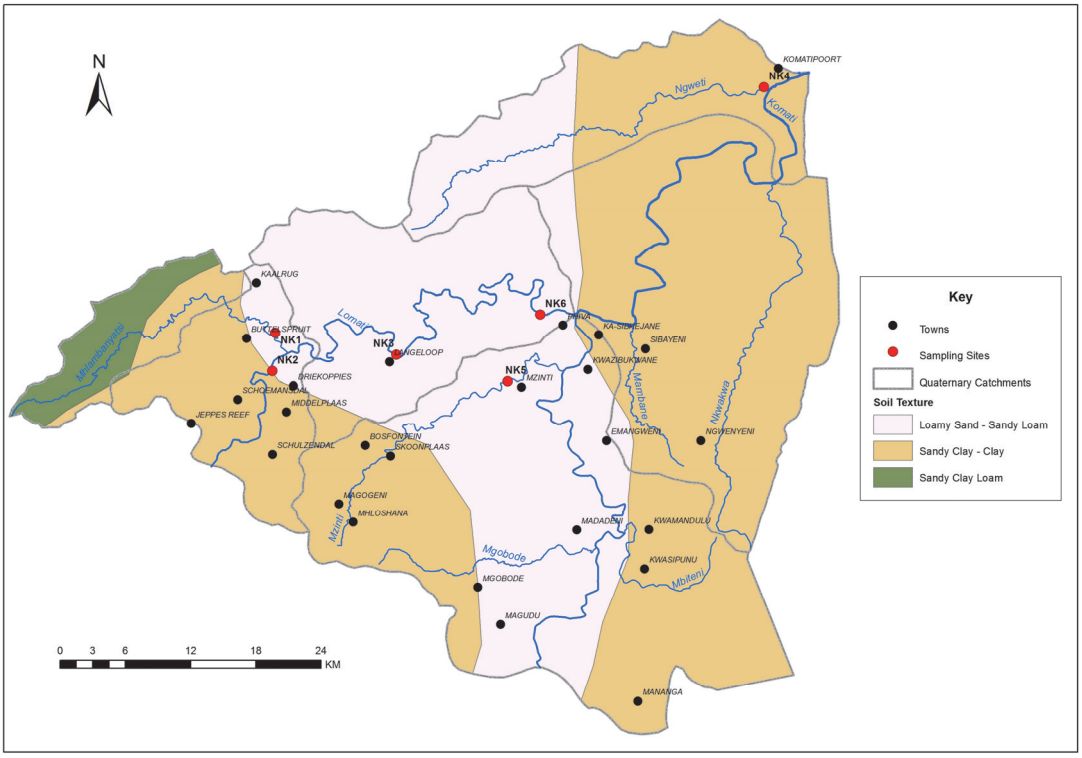

Figure 2.10: Soil texture characteristics of the Lomati, Mzinti and Ngwezi River catchments.

2.5.1 Site Descriptions

Table 2.6: Location of selected study sites in the Lomati, Mzinti and Ngwezi River catchments.

Site	GPS co-ordinates	Brief site description
NK1	-25.652297° 31.541136°	Mhlabanyatsi River, upper Lomati catchments.
NK2	-25.684586° 31.538691°	Upper Lomati River, just below Driekoppies Dam and the town of Schoemansdal.
NK3	-25.671514° 31.639979°	Middle Lomati River, adjacent to Langeloop town, point where water is pumped to Langeloop Water Purification Works
NK3-Tap	-25.672227° 31.639483°	Tap located at Langeloop Water Purification works
NK4	-25.449998° 31.944495°	Lower end of Ngwezi River, downstream of intensive agriculture
NK5	-25.692400° 31.732693°	Lower end of Mzinti River, just upstream of Mzinti town and below Mahushe Shongwe Provincial Reserve.
NK6	-25.636649° 31.760402°	Lower Lomati River at weir just upstream of Phiva town.

2.5.2 Sampling Programme

Table 2.7: Summary of samples collected at seven sites in the Lomati catchment ('X' indicates a sample was collected).

Site	Pesticide Analysis				EDC Bioassays		Inorganic Chemistry
	Screening	Quantitative	Water	Sediment	Water	Sediment	
			June 2012				
NK1	X		X	X	X	X	X
NK2	X		X	X	X	X	X
NK3	X		X	X	X	X	X
NK3 Tap	X		X		X		X
NK4	X		X	X	X	X	X
NK5	X		X	X	X	X	X
NK6	X		X	X	X	X	X
			September & December 2012, March 2013				
NK1		X	X	X	X	X	X
NK2		X	X	X	X	X	X
NK3		X	X	X	X	X	X
NK3 Tap		X	X		X		X
NK4		X	X	X	X	X	X
NK5		X	X	X	X	X	X
NK6	X	X	X	X	X	X	X

In addition three borehole samples (VJK, KOP and KOS) were collected during August 2013. These samples were subjected to the full suite of analyses listed in Table 2.5. VJK was collected from a borehole tap located in a residential household in the town of Viljoenskroon (Renoster catchment). KOP was selected from a farm in the vicinity of the town of Koppies (Renoster catchment). KSD was collected from a farm in the vicinity of Kroonstad. All boreholes were utilised for domestic and drinking water purposes.

CHAPTER 3: AGRICULTURAL PESTICIDES IN WATER AND SEDIMENT

by

J.M. Dabrowski, J.M. Shadung and M. Thwala

CSIR, Natural Resources and Environment, PO Box 395, Pretoria, 0001

3.1 INTRODUCTION

This chapter provides details of the sampling and analysis of pesticide concentrations in water and sediment samples from three study areas selected for this project. Pesticide concentrations in water resources are important input for the risk assessment component of the project. In the context of this study, water resources include both the natural resource (i.e. rivers and dams) as well as water from boreholes and taps used for domestic purposes. Given the number of pesticides registered in South Africa, identification of pesticides for analysis can be very difficult. One approach is to get spray records of applied pesticides in the area. This information is notoriously difficult to get and can vary from one farm to another in a catchment. The approach adopted in this study took advantage of screening analysis in combination with the pesticide prioritisation matrix developed in Deliverable 5 of the project (Chemical Prioritisation - Table 3.1). Screening analysis allows for identification of the presence of in excess of 300 pesticides (appendix 1), but only gives an indication of the presence of a compound (i.e. not its concentration). The screening analysis, together with national information on pesticide use per crop was then used to prioritise specific pesticides for further quantitative analysis – an important step for the risk assessment process. Water and sediment were sampled, as both phases represent and important route of exposure to humans and especially to livestock. Due to high variation in physicochemical properties, different pesticides are often predominantly associated with either the water or sediment phase.

3.2 SAMPLING AND ANALYSIS

Samples were collected according to the methodologies as described in Deliverable 3. At each site, samples were collected according to a quarterly schedule. Screening and quantitative analysis of water and sediment samples was performed by LiquidTech Laboratory using LC/MS/MS techniques. The screening process can identify in excess of 300 current use pesticides. Quantitative analysis was performed for selected pesticides using appropriate standards. See www.liquidtech.co.za for more details.

3.2.1 Extraction

3.2.1.1 Water samples

Samples received were filtered through glass fiber filters to remove particulate matter before being concentrated onto methanol conditioned C18 6 mL solid phase extraction cartridges (Strata, Phenomenex) at a flow rate of 5mL/min. Bound sample was slowly eluted off the dried cartridges using 2 mL methanol followed by 2 mL ethyl acetate. The eluant was vacuum dried (Thermo Scientific Savant Speedvac) until almost dry, and reconstituted in 1 mL purified water.

3.2.1.2 Sediment samples

About 50 g of each received sample was frozen and freeze dried. Five gram of each dried sample was placed inside a cellulose thimble and refluxed with 50 mL methanol for 60 minutes in a Soxhlet apparatus. The solvent was removed under reduced pressure, transferred to a vial and reconstituted in 1 mL methanol.

3.2.2 Analysis

Both water and sediment samples were analysed using an Agilent 1200 SL HPLC with an ABSCIEX 3200 QTRAP hybrid triple quadrupole ion trap mass spectrometer. Two types of analyses were performed depending on whether screening for unknown analytes or performing a targeted analyte quantitation. All data acquisition and processing was performed using Analyst 1.5 (AB SCIEX) software.

3.2.3 Unknown Screens

Twenty microliter of each extracted sample was separated on a C18 (150 mm x 4.6 mm, Gemini NX, Phenomenex) column at a flow rate of 300 uL/min using a 30 min gradient from, in positive ionisation mode, 5% solvent A (H_2O/0.1% formic acid) to 95% solvent B (MeOH/0.1% formic acid) and in negative ionisation mode, 5% solvent A (H_2O/10 mM NH_4OH) to 95% solvent B (MeOH/10 mM NH_4OH) with a total run time of 45 min to allow for column re-equilibration. Eluting analytes were ionised by electrospray in the TurboV ion source with 500^oC heater temperature to evaporate excess solvent, 50 psi nebuliser gas, 50 psi heater gas and 25 psi curtain gas. In positive ionisation mode the ion spray voltage was set at 5500 V and -4500 V in negative ionisation mode.

To analyse the sample for unknown pesticides an MRM-IDA-EPI workflow was followed on the instrument. During a multiple reaction monitoring (MRM) scan type the instrument is used in triple quadrupole mode where every ionised analyte (the precursor) eluting off the column is fragmented in the collision cell to produce fragment masses. A set of masses, the precursor mass and one fragment mass constitutes a transition. The instrument jumps between different transitions in an MRM transition list during an analysis cycle, each cycle typically lasting a few seconds. If a transition is detected the instrument's response is registered and a chromatogram is generated.

When the instrument's response is above a certain threshold value, the instrument switches from triple quadrupole mode to ion trap mode. In a subsequent analysis cycle, the precursor is fragmented, and all fragment masses are recorded, this is referred to as product ion formation. These fragments are collected in the third quadrupole now acting as an ion trap. An enriched spectrum is produced that has all the fragments originating from a specific precursor. The fragmentation profiles of an analyte are unique to that analyte in the way that fingerprints are unique to human individuals. When used to query a spectral library, unknown analytes can be identified with a high level of certainty.

During these screening experiments, the instrument assessed the presence of 271 pesticides. These pesticides were divided into 2 groups depending on their ionisation abilities; 241 in positive ionisation mode and 30 in negative ionisation mode. One MRM for each pesticide was used to, when present, trigger fragmentation and fragment collection. Acquired fragmentation profiles were searched against a library containing MS/MS fragmentation spectra from hundreds of pesticides.

3.2.4 Quantitation

In this approach 20 uL of each extracted sample was separated on a C18 (150mm x 4.6mm, Gemini NX, Phenomenex) column at a flow rate of 300 uL/min using a 2 min gradient from 5% solvent A (H_2O/0.1% formic acid) to 95% solvent B (MeOH/0.1% formic acid) with a total run time of 9 min to allow for column re-equilibration. Eluting analytes were electrospray ionised in the TurboV ion source using 500^{o}C heater temperature to evaporate excess solvent, 50 psi nebuliser gas, 50 psi heater gas and 25 psi curtain gas. Ion spray voltage was set at 5500 V. The targeted analyses of pesticides were performed using 2 MRM transitions per analyte. The peak area on the chromatogram generated from the first and most sensitive transition was used as the quantifier while the second transition is used as a qualifier. The qualifier serves as an additional level of confirmation for the presence of the analyte, the retention time for these two transitions needs to be the same.

3.2.5 Quality control

Samples were submitted into batches that include solvent blank runs between each sample analysed interspersed with quality control samples of known concentration to verify instrument performance. A 4 point calibration curve was generated for each analyte ranging in concentration from 1 ppm to 0.001 ppm with a linear fit through the origin producing a correlation coefficient (*r* value) in excess of 0.98. The limit of detection (LOD) is reported where the signal to noise ratio of the chromatographic peak is above 3 and the lower limit of quantitation (LLOQ) is reported where the signal to noise ratio is above 10. This LLOQ corresponds to about 1µg on column for each analyte.

Table 3.1: Active ingredients and crops prioritized in terms of their potential hazard to human and animal health. Colours represent intensity of use of active ingredient on a crop, from low (dark green) to medium (orange) to high (dark red).

	Rank	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	
	A.I./Crop	Corn	Potatoes	Citrus	Grapes(w)	Sugar Cane	Wheat-winter	Apples	Tomatoes	Grapes(t)	Soybeans	Sorghum	Peaches	Potatoes(s)	Pears	Barley	Sunflower	Beans	Groundnuts	Mangoes	Pineapple	Avocados	Plums	Cotton	Dry-Beans	Apricots	Bananas	
1	Atrazine	87.8	-	-	-	7.5	-	-	-	-	-	4.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
2	Mancozeb	-	15.4	31.1	13.1	-	-	11.7	9.9	2.0	-	-	2.2	4.1	3.3	-	-	1.3	0.9	0.9	0.3	-	0.9	-	-	0.2	0.2	16
3	Acetochlor	84.7	5.7	-	-	1.1	-	-	-	-	5.7	-	-	-	-	-	-	-	2.2	-	-	-	-	0.6	-	-	-	6
4	Ethylene-dibromide	-	97.6	-	-	-	-	-	0.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
5	Terbuthylazine	92.2	-	-	2.9	0.8	-	-	-	1.4	-	2.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5
6	Glyphosate	65.9	-	2.0	8.0	7.2	0.4	2.2	0.0	0.5	7.8	-	1.3	-	1.2	0.4	-	-	-	0.4	0.1	0.1	0.2	-	-	0.6	0.4	18
7	Sulphur	-	-	-	82.6	-	-	-	0.3	11.2	-	-	0.5	-	-	-	-	-	-	2.3	-	-	-	-	-	-	-	5
8	Copper oxychloride	-	5.7	5.5	10.5	-	-	5.1	13.5	0.9	-	-	13.5	4.4	1.5	-	-	6.5	-	11.3	-	8.3	1.0	-	-	1.5	-	14
9	Imidacloprid	11.5	-	68.2	-	-	1.0	5.8	-	-	-	1.0	-	1.9	1.4	-	1.0	-	-	-	-	-	-	-	-	-	-	8
10	Metolachlor	59.0	0.8	-	-	-	2.2	-	-	-	16.6	3.2	-	-	-	-	3.2	1.8	8.1	-	-	-	-	-	5.3	-	-	9
11	2,4-D-amine	59.7	-	-	-	2.9	24.3	-	-	-	-	6.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4
12	Alachlor	41.6	7.6	-	-	8.6	-	-	-	-	14.7	10.1	-	1.5	-	-	14.9	-	0.7	-	-	-	-	-	-	-	-	8
13	MCPA	6.3	1.7	-	7.3	-	54.8	1.5	-	-	-	2.7	0.1	-	1.0	21.8	-	-	-	-	-	-	-	-	-	-	-	9
14	Simazine	68.2	-	4.2	13.8	-	-	0.6	-	12.6	-	-	-	-	0.6	-	-	-	-	-	-	-	-	-	-	-	-	6
15	Paraquat	9.7	10.0	15.6	7.9	12.0	1.8	11.2	3.6	3.1	1.6	-	2.6	-	2.3	1.1	0.7	-	-	0.3	-	1.2	1.1	-	-	0.5	1.4	19
16	Aldicarb	-	80.0	20.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
17	MSMA	-	-	-	-	96.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.5	-	-	-	2
18	Trifluralin	-	-	-	0.4	2.8	22.4	-	0.4	0.4	0.6	-	-	-	-	10.5	35.9	4.2	9.6	-	-	-	-	12.4	-	-	-	11
19	Potassium-phosphite	-	53.8	13.9	4.6	-	-	-	-	4.6	-	-	-	-	-	-	-	-	-	-	23.1	-	-	-	-	-	-	5
20	Diuron	-	-	-	-	100.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
21	Metribuzin	-	8.6	-	-	86.0	-	-	0.5	-	3.6	-	-	1.4	-	-	-	-	-	-	-	-	-	-	-	-	-	5
22	Hexazinone	-	-	-	-	100.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
23	Cyanamide	-	-	-	-	-	-	7.7	-	91.6	-	-	-	-	-	-	-	-	-	-	-	-	0.5	-	-	0.2	-	4
24	Carbofuran	72.8	4.8	-	-	-	-	-	-	-	-	10.0	-	-	-	-	3.8	-	3.8	-	-	-	-	-	-	-	-	5
25	EPTC	54.8	42.0	-	-	-	-	-	-	-	-	-	-	3.2	-	-	-	-	-	-	-	-	-	-	-	-	-	3
26	Fosthiazate	-	72.4	27.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
27	S-metolachlor	47.2	-	-	-	49.6	-	-	-	-	-	3.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
28	Chlorpyrifos	2.5	4.2	-	30.1	-	27.4	11.5	3.4	2.1	3.9	-	2.1	-	12.9	-	-	3.1	-	-	-	-	0.4	-	2.5	-	1.6	14
29	Sulcotrione	95.0	-	-	-	5.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
30	Bromoxynil	17.1	-	-	-	-	57.5	-	-	-	-	1.1	-	-	-	20.3	-	-	-	-	-	-	-	-	-	-	-	4
31	Terbufos	83.7	3.1	12.4	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3	0.5	-	-	-	-	-	-	-	-	5
32	Chlorothalonil	-	54.2	-	-	-	-	-	17.5	-	-	-	-	15.4	-	-	-	3.9	5.6	-	-	-	-	-	-	-	-	5
33	Thiram	-	-	-	-	-	-	0.1	-	-	-	-	66.0	-	-	-	-	-	-	-	-	-	16.1	-	-	14.5	-	4
34	Copper-carbonate	-	-	-	-	-	-	-	34.2	-	-	-	-	32.0	-	-	-	-	-	33.8	-	-	-	-	-	-	-	3
35	Thiamethoxam	84.4	-	-	-	-	7.7	3.3	-	-	-	-	-	-	-	-	3.1	-	-	-	-	-	-	1.5	-	-	-	5
36	Benomyl	9.8	-	46.8	-	-	-	-	8.7	-	8.7	-	-	-	-	-	8.7	-	17.4	-	-	-	-	-	-	-	-	6
37	Copper hydroxide	-	10.6	-	36.4	-	-	-	6.1	-	-	-	-	-	-	-	-	-	-	0.0	-	0.0	-	-	6.1	-	-	6
38	Carbendazim	6.8	-	4.8	0.6	-	51.5	-	-	0.6	-	-	-	-	-	35.7	-	-	-	-	-	-	-	-	-	-	-	6
39	Carbaryl	8.6	-	0.3	25.7	-	-	15.9	-	4.0	-	-	0.4	-	0.4	-	-	-	-	-	-	-	0.2	5.8	1.4	-	2.5	11
40	Iprodione	-	-	-	24.9	-	-	-	20.2	9.2	-	-	8.1	-	31.7	-	-	-	-	-	-	-	1.2	-	-	-	-	6
41	2,4-D	-	-	-	-	100.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
42	Trichlorfon	-	-	100.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
43	Bromacil	-	-	16.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	83.3	-	-	-	-	-	-	2
44	Endosulfan	72.5	-	-	-	-	-	-	11.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
45	Cyproconazole	16.3	-	-	-	-	53.1	-	-	-	-	-	-	-	-	30.6	-	-	-	-	-	-	-	-	-	-	-	3
46	Acephate	-	4.3	-	-	-	-	3.5	15.1	-	-	-	24.3	1.7	0.9	-	-	-	-	-	-	-	-	-	-	-	-	6
47	Dichlorvos	-	-	-	10.0	-	-	-	-	10.0	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3	49.9	-	-	4
48	Buprofenzin	-	-	94.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5.7	-	-	-	-	-	2
49	Tembotrione	100.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
50	Zineb	-	0.9	0.9	-	-	-	-	21.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
51	Triadimenol	-	-	-	16.5	-	37.1	-	-	3.1	-	-	-	-	-	24.7	-	-	-	2.1	-	-	-	-	-	-	-	5
52	Thiodicarb	84.8	-	-	-	-	-	-	-	-	-	6.1	-	-	-	-	-	-	-	-	-	-	-	9.1	-	-	-	3
53	Deltamethrin	36.3	4.8	-	0.5	-	1.0	0.8	3.3	0.8	1.2	0.9	0.3	1.3	0.8	-	0.7	0.8	0.5	-	-	-	-	40.6	1.2	-	-	17
54	Tribenuron-methyl	-	-	-	-	-	42.9	-	-	-	-	-	-	-	-	57.1	-	-	-	-	-	-	-	-	-	-	-	2
55	Triticonazole	-	-	-	-	-	50.0	-	-	-	-	-	-	-	-	50.0	-	-	-	-	-	-	-	-	-	-	-	2
56	Fipronil	-	-	72.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16.9	-	-	-	-	-	-	-	2
57	Triadimefon	-	-	35.7	5.6	-	40.4	1.5	-	4.5	-	-	-	-	-	9.1	-	-	-	0.3	-	-	-	-	-	-	0.9	8
58	Malathion	-	-	100.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
59	Sulfosulfuron	-	-	-	-	-	100.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
60	Beta-cypermethrin	50.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	50.0	-	-	2
61	Thiophanate	-	-	100.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
62	Propoxur	-	-	-	75.0	-	-	-	-	25.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
63	Procymidone	-	20.2	-	9.9	-	-	-	9.9	9.9	-	-	9.9	-	9.9	-	-	20.2	-	-	-	-	-	-	-	-	-	7
64	Bifenthrin	6.7	20.0	-	-	-	-	-	33.3	-	-	-	-	6.7	-	-	-	-	-	-	-	-	-	33.3	-	-	-	5
		29	23	21	21	15	18	15	20	20	10	12	13	11	13	11	9	9	10	10	4	5	9	9	7	6	6	

3.3 LETSITELE CATCHMENT

Sampling in this study area initially took place during July and November 2011. July was characterised by low winter flows, with minimal chance of input of agrochemicals from runoff and leaching processes. Samples collected during November followed the start of the rainy season, when higher flows were experienced.

Samples collected during July were subjected to a screening analysis only. This was to get an idea of which pesticides were present in the catchment and does not provide an indication of concentration, but only of absence or presence. Based on the results of the first trip, together with outputs from the pesticide prioritization process (Table 3.1), specific pesticides were identified for quantitative analysis for samples collected during the second trip in November. Only samples in the main stem of the Letsitele River and community taps were processed for quantitative analysis as these sites were assumed to be more relevant from a human and livestock exposure point of view. In addition, screening analysis was performed at all sites to account for the difference in season and ensure that additional potential high priority pesticides were not excluded from future sampling activities. From August 2012, and every three months thereafter, screening was limited to LT9 (the catchment outlet) as this site was assumed to be the most representative of pesticide use in the catchment. Samples for EDC bioassays were collected concurrently to samples collected for pesticide analysis. An overview of samples collected at each site during the two sampling trips is provided in Table 2.3.

3.3.1 Results

3.3.1.1 Screening Analysis

A total of six pesticides were detected in surface water and sediment samples during the sampling event in July (Table 3.2 and Table 3.3). Diphenylamine and propiconazole were the most commonly detected pesticides and were detected predominantly in the water and sediment phase, respectively. Imidacloprid was detected at two sites, while all other pesticides were detected on only one occasion. Of the samples collected at drinking water points, no pesticides were detected at LT4 and LT5, while diphenylamine was detected at LT6. Pesticides were detected at all of the river sites, and, based on the number of detections of different pesticides, no site appeared to be more contaminated than another.

During the November sampling period carbendazim, flusilazole, prochloraz and sulfamethazine were detected in addition to the five detected during the July sampling period. Again, diphenylamine and propiconazole were detected most regularly, with a fairly equal distribution between the water and sediment phase. Flusilazole and sulfamethazine were only detected on one occasion each. Of the samples collected from drinking water points, LT4 (the tap located at a primary school) showed a high number of detections of different pesticides (five in total). In general sites in the upper section of the Letsitele (LT3 and LT7) recorded a low number of detections with diphenylamine and thiabendazole being detected in the sediment. In general, a greater number of pesticides were detected (at a higher

frequency of detection) during the November sampling period in comparison to July. No pesticides were detected at LT4 and LT9 during August and November 2012. Those pesticides that were analysed quantitatively were detected more frequently in both the water and sediment phases, which justify their prioritization for quantitative analysis.

3.3.1.2 Quantitative Analysis

Quantitative analysis was only performed for pesticides that were detected more frequently in screening analysis. In general, concentrations of all pesticides were highest during the November 2011 sampling event (Table 3.4). Concentrations measured at LT4 (a tap at a local school) were noticeably higher than those detected at other sites. The fact that no pesticides were detected from samples collected at this site during July (dry winter months) indicates that this tap may be linked to a borehole which is periodically contaminated by pesticides during the wet summer months when leaching takes place. This finding illustrates the importance of collecting data and samples over an extended temporal period covering different seasonal weather patterns. At LT6, diphenylamine was detected, although at considerably lower concentrations than at LT4. While there were also a relatively high number of detections during August 2012, the concentrations were mostly lower than those detected during November 2011 and 2012. In general diphenylamine was commonly detected at concentrations considerably higher than for other pesticides. Thiabendazole and imidacloprid were also detected relatively frequently, although at comparatively lower concentrations than diphenylamine. Concentrations of pesticides detected in the sediment were relatively low. While propiconazole was detected frequently during screening assessments, concentrations were below the limits of quantification and results could therefore not be reported.

Table 3.2: Results of pesticide screening analysis conducted on water samples collected from the Letsitele study area (compound coloured in grey were analysed quantitatively). 'X' indicates the pesticide was detected

Date	Site	Diphenylamine	Imizalil	Thiabendazole	Imidacloprid	Propiconazole	Fenuron	Prochloraz	Carbendazim	Flusilazole	Sulfmethazine
Jul-11	LT1				X						
	LT2	X									
	LT3										
	LT4						X				
	LT6	X									
	LT7	X									
	LT8		X	X							
	LT9										
	LT10				X						
Nov-11	LT1	X			X						
	LT2										
	LT3							X			
	LT4	X	X	X		X			X	X	
	LT6										X
	LT7										
	LT8					X					
	LT9	X									
	LT10	X				X					
Aug-12	LT4										
	LT9										
Nov-12	LT4										
	LT9										
Feb-13	LT4										
	LT9										

Table 3.3: Results of pesticide screening analysis conducted on sediment samples collected from the Letsitele study area (compound coloured in grey were analysed quantitatively). 'X' indicates the pesticide was detected

Date	Site	Diphenylamine	Imizalil	Thiabendazole	Imidacloprid	Propiconazole	Fenuron	Prochloraz	Carbendazim	Flusilazole	Sulfmethazine
Jul-11	LT1					X					
	LT10		X			X					
	LT2	X									
	LT3	X				X					
	LT7					X					
	LT8		X			X					
	LT9					X					
Nov-11	LT1					X					
	LT2					X					
	LT3	X									
	LT7			X							
	LT8	X	X	X							
	LT9			X							
Aug-12	LT4										
	LT9										
Nov-12	LT4										
	LT9										

Table 3.4: Concentrations (µg/L) of pesticides detected in water samples collected from selected sites in the Letsitele catchment.

Date	Site	Diphenylamine	Imizalil	Thiabendazole	Imidacloprid	Propiconazole
Nov-11	LT3	0.01	nd	nd	nd	nd
	LT4	0.0272	0.06	0.036	0.003	0.0038
	LT6	0.0006	nd	nd	nd	nd
	LT7	0.002	nd	0.0008	0.002	nd
	LT9	0.003	0.0008	0.0002	0.004	nd
Aug-12	LT10	0.00175	0.0064	0.0007	0.00033	nd
	LT2	nd	nd	0.00009	0.0017	nd
	LT3	nd	nd	0.00009	0.0008	0.0009
	LT4	nd	nd	0.00015	0.0009	nd
	LT9	nd	nd	0.0005	0.002	nd
Nov-12	LT10	nd	nd	nd	0.001	nd
	LT2	nd	nd	nd	nd	nd
	LT3	nd	nd	nd	0.001	0.004
	LT4	nd	nd	nd	nd	nd
	LT9	nd	nd	nd	0.002	nd
Feb-13	LT10	nd	0.0621	0.0045	0.0202	nd
	LT2	nd	nd	nd	nd	nd
	LT3	nd	nd	nd	nd	nd
	LT4	nd	nd	nd	nd	0.0043
	LT9	nd	0.0003	0.00001	0.0043	nd

Table 3.5: Concentrations (µg/kg) of pesticides detected in sediment samples collected from selected sites in the Letsitele catchment**.**

Date	Site	Diphenylamine	Imizalil	Thiabendazole	Imidacloprid	Propiconazole
Nov-11	LT3	0.0000028	nd	0.000018	0.000006	nd
	LT7	nd	nd	0.0000012	nd	nd
	LT9	0.000008	nd	0.00001	nd	nd
Aug-12	LT2	nd	nd	nd	nd	nd
	LT3	nd	nd	0.0003	nd	nd
	LT9	0.001	nd	0.0003	nd	nd
Nov-12	LT2	nd	nd	nd	nd	nd
	LT3	nd	nd	nd	nd	nd
	LT9	nd	nd	nd	nd	nd
Feb-13	LT2	0.00146	nd	0.00013	nd	0.00033
	LT3	nd	nd	0.00014	nd	nd
	LT9	nd	nd	0.00051	nd	nd

Table 3.6: Concentrations (µg/L) of pesticides detected in samples collected from three boreholes (used for drinking water) in the Letsitele catchment.

Site	Diphenylamine	Imizalil	Thiabendazole	Imidacloprid	Propiconazole
SL	nd	0.4910	0.0011	0.0029	nd
FM	nd	nd	0.0003	1.8987	nd
BM	nd	nd	0.0002	0.2765	nd

3.3.2 Summary Statistics

Table 3.7: Summary of pesticides detected in water samples collected from all sites in the Letsitele catchment over the sampling period.

Pesticide	Mean	Median	Max	No. Detections	Detections (%)
Diphenylamine	0.007	0.003	0.027	4	22.2
Imizalil	0.03	0.03	0.06	5	27.8
Thiabendazole	0.004	0.0003	0.036	9	50
Imidacloprid	0.003	0.002	0.02	12	66.7
Propiconazole	0.003	0.004	0.004	4	22.2

Table 3.8: Summary of pesticides detected in sediment samples collected from all sites in the Letsitele catchment over the sampling period.

Pesticide	Mean	Median	Max	No. Detections	Detections (%)
Diphenylamine	0.0006	0.0005	0.0015	4	44.4
Imizalil	nd	nd	nd	nd	nd
Thiabendazole	0.0002	0.0001	0.0003	8	88.9
Imidacloprid	0.000006	0.000006	0.000006	1	11.1
Propiconazole	0.0003	0.0003	0.0003	1	11.1

3.3.3 Link Between Detected Pesticides and the Pesticide/Crop Prioritization Matrix

Of the pesticides detected in the Letsitele catchment only imidacloprid and carbendazim appear on the final list of priority pesticides. Imidacloprid is ranked 9th in the list and citrus accounts for 68% of the total use of this pesticide (Table 3.1). Carbendazim is ranked 38th and is not used widely on citrus. Propiconazole is applied mainly to wheat and barley, however mangoes account for 1.4% of the national use (see Volume 2 of this report). Propiconazole was detected at two sites in the catchment, adjacent to mango orchards. Thiabendazole is applied exclusively to citrus and was detected at all sites in the catchment. Other high priority pesticides applied to these crops but that are not included in the qualitative screen include sulphur, copper oxychloride, mancozeb and glyphosate. These active ingredients were prioritised more on the basis of extensive use as opposed to high toxicity. Diphenylamine was not amongst the list of pesticides included in the pesticide use database. These results indicate the usefulness and potential of the pesticide crop

prioritization matrix as a useful tool to identify potential priority chemicals in an area based on the crops present.

Contamination patterns were also well described by the mobility categories of the respective pesticides, with high and medium category pesticides being detected either more frequently or at higher concentrations than those in the low mobility category (Table 3.7 & Table 3.9). Imidacloprid was the most commonly detected pesticide at relatively high concentrations and fell into a high mobility category (Table 3.7). Furthermore, imidacloprid was detected in all three borehole samples at higher concentrations than the other pesticides. Imazalil, propiconazole and thiabendazole are less mobile and all detected less frequently in water samples. Thiabendazole was however detected more frequently in sediment samples (although at low concentrations), indicating a presence. Sampling results, together with the prioritization outputs would therefore indicate that imidacloprid is the highest priority chemical in this catchment from a water contamination perspective. The presence of diphenylamine cannot be linked to use on crops grown in the area. This pesticide is registered for post-harvest control of scald on apples and pears. There are a number of packing houses in the catchment, although neither apples nor pears are produced in the area.

Table 3.9: Citrus and mango use statistics and mobility categories of pesticides (numbers in parentheses indicate ranking in list of national priority pesticides) detected in the Letsitele River catchment.

Active Ingredient	Area Treated ($x10^3$ ha)	Application rate (kg or L/ha)	Total application ($x10^3$ kg or L)	Mobility
Imidacloprid (9)	81.11	2.120	171.917	High
Carbendazim (38)	3.64	0.200	0.729	Medium
Propiconazole	20.00	0.025	0.500	Medium
Thiabendazole	93.75	0.040	3.750	Low
Imazalil	2.44	0.782	1.908	Low

3.4 VALS AND RENOSTER CATCHMENTS

3.4.1 Results

3.4.1.1 Screening Analysis

Screening was restricted to the lower most sites in each of the rivers (i.e. RN4 and VL3). Initial screening undertaken in February 2012 revealed a high number of compounds (Table 3.10). Of the compounds detected, atrazine, simazine, terbuthylazine, imidacloprid and alochlor were identified as relatively high priority during the prioritization process and were selected for further quantitative analysis. These pesticides were detected relatively frequently in subsequent sampling, while all other agricultural pesticides identified by screening were detected relatively less frequently, with the exception of tebuthiuron, terbutryn and prometon. The majority of

pesticides detected were herbicides (e.g. atrazine, simazine, terbuthylazine, alachlor and metolachlor).

3.4.1.2 Quantitative Analysis

Atrazine and terbuthylazine were detected in all water samples collected during the sampling period Table 3.12. In comparison to concentrations of pesticides detected in the Letsitele catchment, concentrations were markedly higher in the Vals and Renoster catchments. There was no obvious difference in the magnitude of concentrations of these two compounds in samples collected during July 2012 when compared to those collected in October 2012. Simazine, while not detected as frequently, was also detected at comparatively high concentrations. There was no obvious trend in pesticide concentrations from upstream to downstream in the Vals River. In contrast, concentrations of atrazine and terbuthylazine were consistently lower at RN1 than detected at RN3 and RN4. Alachlor was detected less frequently at comparatively lower concentrations levels. Imidacloprid was detected on only one occasion at VL2.

Similar patterns of contamination were evident in sediment samples, with atrazine and terbuthylazine being the most frequently detected (Table 3.13). Concentrations were however comparably lower than those observed in the water phase.

Table 3.10: Results of pesticide screening analysis conducted on water samples collected from the Vals and Renoster catchments (compounds coloured in grey were analysed quantitatively). 'X' indicates the pesticide was detected ([1]Breakdown product of atrazine; [2]Breakdown product of tebuthylazine).

Date	Site	Alachlor	Atrazine	Imidacloprid	Simazine	Terbuthylazine	Popazyn-2-hydroxy	Tebuthiuron	Metolachlor	Terbutryn
Feb-12	RN4	X		X	X	X				
	VL3		X		X	X		X	X	X
Jul-12	RN4		X			X	X	X	X	X
	VL3		X		X	X		X		X
Oct-12	RN4		X			X				
	VL3				X	X		X		
Jan-13	RN4		X			X		X		
	VL3		X		X	X				X
Apr-13	RN4		X			X		X		X
	VL3		X		X	X				X

Table 3.10 (cont):

Date	Site	Prometon	Diphenylamine	Cyanazine	Ametryn	Carbendazim	Thiabendazole	Propazine	Atrazine-diesypropyl[1]	Tebuthylazine-desethyl[2]
Feb-12	RN4					X	X	X	X	X
	VL3	X			X				X	X
Jul-12	RN4								X	
	VL3	X								X
Oct-12	RN4		X	X						
	VL3	X	X							
Jan-13	RN4									
	VL3									
Apr-13	RN4									X
	VL3	X							X	

Table 3.11: Results of pesticide screening analysis conducted on sediment samples collected from the Vals and Renoster catchments (compounds coloured in grey were analysed quantitatively). 'X' indicates the pesticide was detected.

Date	Site	Alachlor	Atrazine	Imidacloprid	Simazine	Terbuthylazine	Popazyn-2-hydroxy	Tebuthiuron	Metolachlor	Terbutryn
Jul-12	RN4					X				
	VL3									
Oct-12	RN4					X				
	VL3									
Jan-13	RN4									
	VL3									
Apr-13	RN4									
	VL3									

Table 3.11 (cont.):

DATE	SITE	Prometon	Diphenylamine	Cyanazine	Ametryn	Carbendazim	Thiabendazole	Propazine	Atrazine-diesypropyl[1]	Tebuthylazine-desethyl[2]
Jul-12	RN4		X							
	VL3		X							
Oct-12	RN4		X							
	VL3		X							
Jan-13	RN4									
	VL3									
Apr-13	RN4									
	VL3									

[1]Breakdown product of atrazine
[2]Breakdown product of tebuthylazine

Table 3.12: Concentrations (µg/L) of pesticides detected in water samples collected from selected sites in the Vals and Renoster catchments

Date	Site	Alachlor	Atrazine	Imidacloprid	Simazine	Terbuthylazine
Jul-12	RN1	nd	0.006	nd	nd	0.008
	RN3	nd	0.033	nd	nd	0.048
	RN4	0.002	0.077	nd	nd	0.077
	VL1	nd	0.0355	nd	nd	0.0413
	VL2	nd	0.012	nd	0.03	0.015
	VL3	nd	0.0177	nd	0.037	0.012
Oct-12	RN1	0.0052	0.0073	nd	nd	0.0095
	RN3	nd	0.044	nd	nd	0.0612
	RN4	nd	0.0695	nd	nd	0.0518
	VL1	nd	0.0254	nd	nd	0.0161
	VL2	nd	0.0399	nd	0.0789	0.03
	VL3	nd	0.0512	nd	0.0669	0.0348
Jan-13	RN1	nd	0.042	nd	0.068	0.023
	RN3	nd	0.275	nd	0.15	0.137
	RN4	nd	0.092	nd	0.085	0.047
	VL1	nd	0.048	nd	0.115	0.037
	VL2	nd	0.35	nd	0.9	0.158
	VL3	nd	0.102	nd	0.0007	0.041
Apr-13	RN1	nd	0.0105	nd	nd	0.0091
	RN3	nd	0.0112	nd	nd	0.0656
	RN4	nd	0.0448	nd	nd	0.0346
	VL1	nd	0.1649	nd	nd	0.1031
	VL2	0.002	0.1752	0.0196	0.2902	0.2074
	VL3	nd	0.1176	nd	0.3732	0.0865

Table 3.13: Concentrations (µg/kg) of pesticides detected in sediment samples collected from selected sites in the Vals and Renoster catchments.

Date	Site	Alachlor	Atrazine	Imidacloprid	Simazine	Terbuthylazine
Jul-12	RN1	nd	nd	nd	nd	nd
	RN3	nd	nd	nd	nd	0.001
	RN4	nd	0.004	nd	nd	0.009
	VL1	nd	nd	nd	nd	nd
	VL2	nd	0.001	nd	0.001	0.003
	VL3	nd	nd	nd	nd	nd
Oct-12	RN1	nd	0.001	nd	nd	0.002
	RN3	nd	0.001	nd	nd	0.002
	RN4	nd	0.001	nd	nd	0.001
	VL1	nd	nd	nd	nd	nd
	VL2	nd	0.001	nd	0.001	0.002
	VL3	nd	nd	nd	nd	nd
Jan-13	RN1	nd	0.00037	nd	nd	0.0007
	RN3	nd	0.00048	nd	nd	0.00073
	RN4	nd	0.00023	nd	nd	0.00025
	VL1	nd	nd	nd	nd	0.0004
	VL2	nd	0.0006	nd	nd	0.00131
	VL3	nd	0.00023	nd	nd	0.00022
Apr-13	RN1	nd	nd	nd	0.00053	0.00025
	RN3	0.00165	0.00046	nd	nd	0.00049
	RN4	nd	0.00019	nd	0.00144	0.00035
	VL1	nd	0.00031	nd	nd	0.00043
	VL2	nd	0.00048	nd	nd	0.00186
	VL3	nd	0.00133	nd	nd	0.00161

Table 3.14: Concentrations (µg/L) of pesticides detected in samples collected from three boreholes (used for drinking water) in the Vals and Renoster catchments.

SITE	Alachlor	Atrazine	Imidacloprid	Simazine	Terbuthylazine
VJK	nd	0.0212	nd	0.0296	0.0011
KSD	nd	0.0059	nd	nd	0.0006
KOP	nd	0.0005	nd	nd	0.0003

3.4.2 Summary Statistics

Table 3.15: Summary of pesticides detected in water samples collected from all sites in the Vals and Rensoter catchments over the sampling period.

Pesticide	Mean	Median	Max	No. Detections	Detections (%)
Alachlor	0.003	0.002	0.002	3	12.5
Atrazine	0.11	0.072	0.275	24	100
Imidacloprid	0.0196		0.02	1	4.2
Simazine	0.18	0.08	0.15	12	50
Terbuthylazine	0.056	0.041	0.137	24	24

Table 3.16: Summary of pesticides detected in sediment samples collected from all sites in the Vals and Rensoter catchments over the sampling period.

Pesticide	Mean	Median	Max	No. Detections	Detections (%)
Alachlor	0.002	0.002	0.002	1	4.1
Atrazine	0.0009	0.0005	0.004	16	66.7
Imidacloprid	nd	nd	nd	nd	nd
Simazine	0.001	0.001	0.001	4	16.7
Terbuthylazine	0.002	0.001	0.009	19	79

3.4.3 Link Between Detected Pesticides and the Pesticide/Crop Prioritization Matrix

According to the pesticide crop prioritization matrix, atrazine, simazine, terbuthylazine, alachor and metolachlor are all predominantly used on maize (accounting for in excess of 40% of the use of each pesticide) and feature relatively high up in the national priority list of pesticides, falling within the top 16 priority compounds identified in the national prioritization procedure. Furthermore, the most commonly detected pesticides (atrazine, simazine and terbuthylazine; Table 3.15) were all categorized as having high mobility potential (Table 3.17). The fact that these herbicides were detected throughout the year (during the wet and dry season) indicates that they have essentially saturated the hydrological cycle. This is most likely due to their high mobility in combination with their high quantities of use.

Of the pesticides applied to maize that occur in the list of the top 20 national priority pesticides only paraquat, MCPA, 2,4D-amine and chlorpyrifos were not detected. Of these, paraquat and glyphosate are not included in the list of analytes included in the screening procedure. Imidacloprid and carbendazim are also used on maize although not as extensively as the above listed pesticides (imidacloprid is widely used at very low rates of application while carbendazim is not widely used on maize - Table 3.17). According to the matrix, ametryn and thiabendazole are used on sugar cane and citrus, respectively, both of which are produced in small quantities in the catchment area. Therefore, the pesticide/crop prioritization matrix provided an excellent reflection of pesticides detected in the catchment area. A number of pesticides

detected by screening analysis were not listed as being used on any crop in South Africa. These include propazyn-2-hydroxyl, tebuthiuron, terbutryn, prometon, cyanazine and propazine. Tebuthiuron is commonly used to control bush encroachment and to keep paved roads, railways and fence-lines permanently devoid of vegetation. Prometon is also registered for use for total vegetation control in non-agricultural areas.

Table 3.17: Maize use statistics and mobility categories of pesticides (numbers in parentheses indicate ranking in list of national priority pesticides) detected in the Vals and Renoster rivers.

Active Ingredient	Area Treated ($x10^3$ ha)	Application rate (kg or L/ha)	Total application ($x10^3$ kg or L)	Mobility
Atrazine (1)	951.89	0.936	890.981	High
Terbuthylazine (5)	712.64	0.873	621.787	High
Imidacloprid (9)	661.79	0.044	28.953	High
Alachlor (12)	79.00	1.513	119.520	Medium
Simazine (14)	69.32	0.819	56.753	High
Carbendazim	8.20	0.125	1.025	Medium
Ametryn	No estimated use on maize			Medium
Thiabendazole	No estimated use on maize			Low

3.5 LOMATI CATCHMENT

3.5.1 Results

3.5.1.1 Screening Analysis

Screening analysis was conducted on all samples collected during the June and September 2012 sampling trips (Table 3.18). Initial sampling performed in June revealed no detections of any pesticides. This is most likely as a result of it being the dry season, with minimal potential input as a result of runoff or leaching. Sampling during September 2012 took place soon after the first heavy rains of the rainy season and these samples had a high number of detections of a number of different pesticides. Pesticides selected for further quantitative analysis included atrazine, terbuthylazine, imidacloprid, carbofuran and thiabendazole. These pesticides were all detected relatively frequently during the September 2012 screening analysis. Additional pesticides that were also detected relatively frequently across sampling sites included ametryn, diuron and hexazinone. These pesticides were not analysed quantitatively due to standards not being available. Their relatively frequent detection would however warrant them being included in future monitoring programmes.

From December 2012 screening was only conducted at NK6, as this is the most downstream site in the Lomati River catchment and would therefore be representative of the majority of pesticide use occurring in the catchment. Screening

analysis during December 2012 revealed far less detection of different pesticides than from the same site during September 2012.

Table 3.18: Results of pesticide screening analysis conducted on water samples collected from the Lomati, Mzinti and Ngwezi River catchments (compound coloured in grey were analysed quantitatively). 'X' indicates the pesticide was detected.

Date	Site	Atrazine	Imidacloprid	Terbuthylazine	Thiabendazole	Carbofuran	Diuron	Hexazinone	Ametryn	Diphenylamine	Prometryn	Metribuzin	Metolachor
Jun-12	NK1												
	NK2												
	NK3												
	NK3-tap												
	NK4												
	NK5												
	NK6												
Sep-12	NK1	X		X									
	NK2								X				
	NK3		X	X			X	X	X		X		
	NK3-tap	X	X	X			X	X					
	NK4	X	X	X	X	X	X		X		X	X	X
	NK5	X		X									
	NK6	X	X	X		X	X		X				
Dec-12	NK6	X		X					X				
Mar-13	NK3												
	NK6												

Table 3.19: Results of pesticide screening analysis conducted on sediment samples collected from the Lomati, Mzinti and Ngwezi River catchments (compound coloured in grey were analysed quantitatively). 'X' indicates the pesticide was detected.

Date	Site	Atrazine	Imidacloprid	Terbuthylazine	Thiabendazole	Carbofuran	Diuron	Hexazinone	Ametryn	Diphenylamine	Prometryn	Metribuzin	Metolachor
Jun-12	NK6								X	X			
Sep-12	NK6								X				
Dec-12	NK6												
Mar-13	NK6								X				

3.5.1.2 Quantitative Analysis

No pesticides were detected during the June 2012 sampling trip. Atrazine and terbuthylazine were detected in all samples collected for all sites during both September and December 2012 (Table 3.20). There was no obvious difference in the magnitude of concentration levels between the two sampling periods. Carbofuran was also detected (at comparatively low concentrations) across most sites during the latter two sampling periods (with the exception of NK5). Concentrations measured in samples collected during December 2012 were generally approximately an order of magnitude lower than those measured in samples collected during September 2012.

Table 3.20: Concentrations (µg/L) of pesticides detected in water samples collected from selected sites in the Lomati, Mzinti and Ngwezi River catchments.

Date	Site	Atrazine	Imidacloprid	Terbuthylazine	Thiabendazole	Carbofuran
Jun-12	NK1	nd	nd	nd	nd	nd
	NK2	nd	nd	nd	nd	nd
	NK3	nd	nd	nd	nd	nd
	NK3-tap	nd	nd	nd	nd	nd
	NK4	nd	nd	nd	nd	nd
	NK5	nd	nd	nd	nd	nd
	NK6	nd	nd	nd	nd	nd
Sep-12	NK1	0.008	nd	0.0037	nd	nd
	NK2	0.0011	nd	0.0037	nd	0.0003
	NK3	0.0093	0.0431	0.0038	0.0001	0.005
	NK3-tap	0.0083	0.0411	0.0034	0.0001	0.0042
	NK4	0.0926	0.0135	0.011	0.0069	0.0561
	NK5	0.0032	nd	0.0007	nd	nd
	NK6	0.018	0.0647	0.0079	0.0001	0.0071
Dec-12	NK1	0.02	nd	0.004	nd	0.001
	NK2	0.003	nd	0.005	nd	0.0003
	NK3	0.01	nd	0.004	nd	0.0006
	NK3-tap	0.008	nd	0.002	nd	0.0003
	NK4	0.192	nd	0.02	nd	0.0008
	NK5	0.005	nd	0.003	nd	nd
	NK6	0.01	nd	0.004	nd	0.0003
Mar-13	NK1	0.0036	0.0005	0.0007	nd	0.0001
	NK2	0.0027	0.0023	0.0013	nd	0.0003
	NK3	0.0052	0.0038	0.0032	0.0044	0.0004
	NK3-tap	0.0047	0.0034	0.0025	0.0001	0.0003
	NK4	0.0063	0.0279	0.0011	nd	0.0002
	NK5	nd	nd	0.0004	nd	0.00002
	NK6	0.0038	0.002	0.0028	nd	0.0003

Imidacloprid and thiabendazole were both detected in the middle to lower reaches of the Lomati River and in the Ngwezi River. These pesticides were only detected in samples collected during September 2012 and no concentrations were measured in samples collected during the December 2012 sampling trip. Concentrations of imidacloprid were relatively high in comparison to all other pesticides that were quantitatively analysed. In contrast thiabendazole was detected at relatively low concentrations in comparison to other pesticides that were quantitatively analysed.

NK4 in particular showed relatively higher concentrations of atrazine, terbuthylazine, carbofuran and thiabendazole when compared to other sites. There was a direct link between pesticides quantified in samples collected from the tap at the Langeloop Water Purification Plant and those collected from site NK3. First of all pesticides detected at NK3 were always detected in samples collected from the tap. Secondly, concentrations of the detected pesticides were always slightly lower in samples collected from the tap in comparison to those collected from the river. These results indicate that concentrations of pesticides occurring in the Lomati River are only slightly reduced by the treatment process at the purification works and are distributed to domestic water supply networks throughout the town of Langeloop.

Table 3.21: Concentrations (µg/kg) of pesticides detected in sediment samples collected from selected sites in the Lomati, Mzinti and Ngwezi River catchments**.**

Date	Site	Atrazine	Imidacloprid	Terbuthylazine	Thiabendazole	Carbofuran
Jun-12	NK1	0.001	nd	nd	nd	nd
	NK2	nd	nd	nd	nd	nd
	NK3	nd	nd	nd	nd	nd
	NK5	nd	nd	nd	nd	nd
	NK6	nd	nd	nd	nd	nd
Sep-12	NK1	0.001	nd	nd	nd	nd
	NK2	nd	nd	0.005	nd	nd
	NK3	0.001	nd	nd	0.0002	nd
	NK4	0.003	nd	0.0005	nd	nd
	NK5	nd	nd	nd	nd	nd
	NK6	0.001	nd	nd	nd	nd
Dec-12	NK1	0.00023	nd	nd	nd	nd
	NK2	nd	nd	nd	0.00012	nd
	NK3	0.00043	nd	0.00022	nd	nd
	NK4	0.00101	nd	0.00015	0.00022	0.00015
	NK5	nd	nd	nd	nd	nd
	NK6	nd	nd	nd	nd	nd
Mar-13	NK1	nd	nd	nd	nd	nd
	NK2	0.00014	nd	nd	nd	nd
	NK3	0.00027	nd	nd	nd	nd
	NK4	0.00106	nd	0.00027	0.0003	0.00048
	NK5	nd	nd	nd	nd	nd
	NK6	0.00048	nd	0.00036	0.0001	nd

In general the pesticides selected for quantitative analysis were detected less frequently and at lower concentrations in the sediment than in the water phase (Table 3.22 & Table 3.23). Atrazine and terbuthylazine were detected most frequently in sediment samples, while imidacloprid and carbofuran were detected on very few occasions (or not at all).

No pesticides were detected in any of the borehole samples collected from the study area.

3.5.2 Summary Statistics

Table 3.22: Summary of pesticides detected in water samples collected from all sites in the Lomati catchment over the sampling period.

Sites	Mean	Median	Max	No. Detections	Detections (%)
Atrazine	0.021	0.007	0.19	20	71.4
Imidacloprid	0.02	0.009	0.065	10	35.7
Terbuthylazine	0.004	0.003	0.02	21	75
Thiabendazole	0.0002	0.0001	0.007	6	21.4
Carbofuran	0.004	0.0003	0.056	18	64.3

Table 3.23: Summary of pesticides detected in sediment samples collected from all sites in the Lomati catchment over the sampling period.

Sites	Mean	Median	Max	No. Detections	Detections (%)
Atrazine	0.0009	0.001	0.003	12	52.2
Imidacloprid	nd	nd	nd	nd	nd
Terbuthylazine	0.001	0.0003	0.005	6	26
Thiabendazole	0.0002	0.0002	0.0003	5	21.7
Carbofuran	0.0003	0.0003	0.0005	2	8.7

3.5.3 Link Between Detected Pesticides and the Pesticide/Crop Prioritization Matrix

In general there was a good link between the crop prioritization matrix and those pesticides detected across the study sites (Table 3.22). Atrazine, terbuthylazine, ametryn, diuron, metribuzin and hexazinone were all identified as being applied to sugar cane. According to the matrix, ametryn, diuron, metribuzin and hexazinone are applied almost exclusively to sugar cane. All of the above mentioned pesticides (with the exception of ametryn) fall within the top 22 of nationally prioritised pesticides. Their relatively frequent detection can be explained by their high usage as well as their generally high mobility (Table 3.24). Imidacloprid and thiabendazole are applied extensively to citrus, with the former pesticide being positioned relatively high in the list of priority pesticides. Imidacloprid in particular is also relatively mobile. Carbofuran was not identified as being applied to either sugar cane or citrus, but

together with metolachlor (which was detected on one occasion) are both applied on other crops (predominantly maize, as well as potatoes, sorghum and sunflower) and their detection indicates the presence of other crop types apart from sugar cane and citrus. The relative high frequency of detection of carbofuran is indicative of its high mobility. Pesticides that were detected and not included in the list of pesticides applied to crops in South Africa include diphenylamine and prometryn.

Table 3.24: Sugar cane and citrus use statistics and mobility categories of pesticides (numbers in parentheses indicate ranking in list of national priority pesticides) detected in the Lomati River catchment.

Active Ingredient	Area Treated ($x10^3$ ha)	Application rate (kg or L/ha)	Total application ($x10^3$ kg or L)	Mobility
Atrazine (1)	50.12	1.515	75.924	High
Terbuthylazine (5)	33.33	0.169	5.625	High
Metolachlor (10)	No estimated use on sugar cane or citrus			Medium
Imidacloprid (9)	661.79	0.044	28.953	High
Diuron (20)	43.37	2.213	96.000	Medium
Metribuzin (21)	63.33	1.440	91.200	Medium
Hexazinone (22)	32.31	2.618	84.607	High
Carbofuran (24)	No estimated use on sugar cane or citrus			High
Ametryn	65.06	1.227	79.810	Medium
Thiabendazole	93.75	0.040	3.750	Low

3.6 RECOMMENDATIONS FOR FUTURE RESEARCH

Information on crop specific pesticide use and mobility (as determined by physicochemical properties obtained from internet databases) were useful for identifying specific pesticides likely to be detected in water resources in the catchment areas of interest. These information resources, together with the pesticide use maps (see Volume 2 of the report) should be consulted when planning and executing studies on the risk of pesticides in South African water resources. Furthermore, the usefulness of the crop specific pesticide use data highlights the need to regularly provide and disseminate updated data of pesticide use in the country. These data resources are critical to the design of monitoring programmes and identification of hotspot areas susceptible to high pesticide contamination.

One of the primary aims of this monitoring exercise was to define an exposure profile for the different pesticides in the study areas for use in the human health risk assessment. Monitoring of field relevant pesticide concentrations is often problematic due to the transient nature of contamination. Pesticides enter rivers as a result of runoff and spray drift and peak, toxicologically relevant concentrations are therefore only present for a relatively short period of time. Conventional monitoring approaches (monthly sampling) will therefore often miss these critical exposure periods, resulting in uncertainty related to the frequency, magnitude and duration of exposure for risk assessment.

Given the financial and logistical implications associated with a monitoring programme with increased frequency of sampling, future research should focus on the use of passive samplers for pesticide monitoring studies. Passive samplers are devices that can be left in the water resource for a prolonged period of time and accumulate pesticide levels for the duration of their deployment. They are therefore able to capture important exposure periods that would otherwise be missed by a routine monitoring programme and can provide a time integrated measurement of exposure covering the period of their deployment. Passive samplers have historically been used to monitor more hydrophobic compounds (e.g. persistent organic pollutants or POPs), however advances in the field have resulted in samplers being developed that can monitor current use pesticides (Schäfer *et al*., 2008; Shaw *et al*., 2010). They are relatively cheap and easy to prepare, yet, to our knowledge, no studies in South Africa have investigated their use in monitoring current use pesticides. This provides an ideal opportunity to advance the field and help improve pesticide monitoring capabilities in South Africa. In addition, catchment scale modelling approaches can also provide supplementary data to constrained monitoring programmes, and particularly within the context of a resource constrained country like South Africa, should be prioritised for future research.

While the analytical approach adopted in this study catered for a large number of different pesticides it is important to note that glyphosate was not included in screening or quantitative analysis; this despite the fact that glyphosate is the most heavily applied pesticide in South Africa (maize accounts for approximately 66% of its total use) and throughout many other parts of the world. Glyphosate is often excluded from monitoring programmes due to difficulties in quantifying this polar and water soluble compound at environmentally relevant concentrations (Battaglin *et al.*, 2014; at the time of the inception of this project, no laboratory that the project team consulted could analyse glyphosate). Glyphosate is generally regarded as having low environmental mobility, due to its rapid breakdown and its high tendency to adsorb to soil (Carlisle and Trevors, 1988). Studies have however shown that glyphosate and its breakdown product, aminomethylphosphonic acid (AMPA), are widely and regularly detected in the environment (Coupe *et al*., 2012; Battaglin *et al.*, 2014). The half-life of glyphosate has been shown to vary widely (from a few days to years) and adsorption to soil is dependent to a large extent on the soil properties (e.g. organic carbon, pH, mineral composition, phosphate competition etc.) to the extent that the use of K_{OC} is regarded as a poor indicator of soil sorption (Vereecken, 2005; Borgaard and Gimsing, 2008). AMPA is generally more persistent in the environment and shows similar toxic effects to its parent compound. While leaching is not a primary route of contamination, groundwater resources have been shown to be contaminated, primarily due to preferential flow (e.g. via macropores in the soil structure, Vereecken, 2005). Runoff shortly after application has been shown to result in input of glyphosate into surface waters (Borgaard and Gimsing, 2008). While the toxicity of glyphosate has generally been regarded as being very low, there is increasing evidence to suggest that glyphosate and AMPA is toxic and an endocrine disruptor (Gasnier *et al.*, 2009). Considering its high quantity of use as well as increasing evidence of human health related effects, future research should focus on developing analytical methods for detection of this pesticide (and its breakdown products) in water resources in South Africa.

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CHAPTER 4: AIR DISPERSION MODELLING OF PESTICIDES

by

P.B.C. Forbes, B.A. Castleman and S.A. Nsibande

Department of Chemistry, Faculty of Natural & Agricultural Sciences, University of Pretoria, Pretoria, 0002

4.1 INTRODUCTION

Loss of agrichemicals, specifically pesticides, to the atmosphere due to spray drift, evaporation and wind erosion with deposition away from the intended site of application, is problematic due to the potential environmental contamination which can result, including pollution of water resources. In addition, off-target sites may be damaged by these chemicals and the resulting lower rate of application on the target site than intended or required may reduce the effectiveness of the pesticide. Besides environmental degradation, there are also financial costs incurred due to these losses.

In Europe, over 80 current-use pesticides have been detected in rain and 30 in ambient air. The compounds which have been studied and detected most frequently include the organochlorine insecticide, lindane, and triazine herbicides, especially atrazine. Acetanilide and phenoxyacid herbicides, as well as organophosphorus insecticides have also been frequently found in rain and air samples (Van Dijk and Guicherit, 1999). Elevated concentrations of pesticides in rain and air are usually only found during the application season, although the less volatile and more persistent pesticides, such as lindane and triazines, are often present at low concentrations throughout the year (Van Dijk and Guicherit, 1999 and Siebers *et al.*, 1994).

Concentrations of pesticides in air usually range from a few pg/m^3 to many ng/m^3, whilst concentrations in rain range from a few ng/L to several µg/L. Deposition rates have been found to vary between a few mg/ha/y to more than 1 g/ha/y (Van Dijk and Guicherit, 1999).

During pesticide application a significant amount, up to 50%, of the applied pesticide can drift more than 300 m off the target crop (Maybank *et al*., 1978; Salyani and Cromwell, 1992; van den Berg *et al*., 1999). Although most pesticides are specifically designed to have low persistence in the environment, they still undergo atmospheric transport at regional or global scales. For example, triazines, acetanilides, phenoxyacids and others are sufficiently stable to undergo atmospheric transport over 100-1000 km (van Dijk and Guicherit, 1999). Once they enter the atmosphere, pesticides can undergo both regional (Muir *et al*., 2004) and long range (Oehme, 1991) transport. A study by Murray and Vaughan (1970) measured pesticide drift up to over 6 km away from the application site. Air currents can transport pesticides over thousands of kilometers to areas as remote as the polar regions where no agricultural activities take place (Unsworth *et al*., 1999).

Pesticide spray drift is of environmental concern because it can lead to serious environmental contamination and is a potential hazard (Murray and Vaughan, 1970).

Studies have suggested that spray drift is one of the important routes for pesticide contamination of natural surface waters and off-crop habitats (Dabrowski and Schulz, 2003; Wang and Rautmann, 2008). Pesticide contamination can compromise the water quality and have negative effects on aquatic life, for example, studies have shown that some of the pesticides may have endocrine disrupting effects to some animals (Hass *et al*., 2012; Hrouzková and Matisová, 2012; Kojima *et al*., 2004). Pesticide mixtures in water can also interact synergistically thereby causing enhanced toxicity to aquatic life (Norgaard and Cedergreen, 2010). Since pesticide spray drift is a significant route for contamination, it is important to monitor the atmospheric dispersion of pesticides during application.

There are a vast number of variables which determine the extent of spray drift, as well as the rates of volatilisation and deposition of agricultural chemicals, and some of these are discussed in the following sections. One of the main factors which prevents the estimation or modelling of these mechanisms is the fact that pure substances (with defined physico-chemical properties) are not used in practise, but that formulations are applied which may vary considerably in composition. This is due to the use of adjuvants, which are substances other than water that enhance the effectiveness of the pesticide with which they are used. Adjuvants for use with agricultural pesticides include extenders, wetting agents, sticking agents and fogging agents.

4.1.1 Methods of Pesticide Application

Despite research into alternative methods, chemical control is still the major method of controlling most weeds, insects and other pests. Methods of pesticide application vary according to the pest that is being targeted, as well as the crop that is being protected, and pesticide spray drift is known to vary with the application method. Pesticides can be applied directly onto the pest or onto the plants or soil. Different formulations, dry, liquid or aerosol, are available depending on the application needs, i.e. application methods or equipment.

Dry formulations include dusts, granules, wettable and soluble powders, as well as water dispersible granules. Dusts are of a small particle size which makes them susceptible to wind drift as well as being more easily “blown off” plants or other surfaces. Granules are larger particles (100-2500 μm), and are often used for application to soils.

Liquid formulations may be in the form of solutions, emulsions, aerosols or fumigants. Solutions may be made with water or organic solvents. Emulsifiable concentrates allow non-water soluble pesticides to be mixed with water in the field. Aerosol formulations consist of the pesticide in a solvent together with a propellant, and are used for fogging or misting, with droplet size dependent on target pest or application.

There are various methods utilised in the application of pesticides, including:

- Hand-operated hydraulic sprayers
- Power-operated hydraulic sprayers
- Air-carrier sprayers

- Controlled droplet application
- Fogging
- Dust and granule application
- Aerial application
- Injection and fumigation techniques.

All sprayers consist of three basic components:

(i) A tank or container to hold the liquid spray.

(ii) A pump or gravity feed system to move the liquid from the tank.

(iii) One or more outlets called nozzles.

There are no universal nozzles; different designs are used to achieve the required range of droplet sizes. As energy is required to break up the liquid into droplets, nozzles are generally classified according to the energy used, i.e. hydraulic, gaseous, centrifugal, kinetic and thermal (Matthews, 1979).

Hydraulic energy nozzles are used the most extensively as their outputs and spray patterns are flexible, with flat-fan and hollow-cone nozzles being the most common. The flat-fan nozzles are available in a range of sizes, producing sprays that vary from very fine to coarse. Movement of droplets after release from a nozzle depends on their size, the wind velocity and direction, and the height of the release above crop or ground. Large numbers of small droplets produced by hydraulic nozzles can become influenced by atmospheric air movements resulting in spray drift and inadequate targeting.

Tractor mounted boom sprayers can be used to supply 50-500 L/ha (Matthews, 1979). Self-propelled sprayers are mainly used on farms with enough flat land to allow the use of booms of up to 27 m width and where the capital outlay can be justified. Variation in spray deposit becomes a possibility when booms are lengthened, due to vertical bounce and/or horizontal whip, which increase with increasing boom width. The nozzles on the spray boom depend on the material being sprayed, the volume of liquid needed and the target to ensure that output, spray pattern and angle, as well as droplet size are appropriate. Cone nozzles are preferred for application of insecticides and herbicides to foliage whereas fan nozzles are usually used when treating the soil. Spray angle and pattern are affected by pressure. To reduce drift, herbicides are typically sprayed at low pressure (0.7-1.0 bar) (Matthews, 1979).

Aircraft are able to apply pesticides rapidly over large areas especially when crop height and waterlogged areas restrict the use of wheeled vehicles, and aerial applications do not contribute to soil compaction and structural breakdown. To minimise drift, pesticides applied by air must be in the form of medium to large droplets.

The swath width or aircraft track spacing is another important practical parameter. The choice of swath width will depend on the behaviour of the pest and the type of foliage of the host crop, as well as the wind velocity. Insects exposed on the tops of plants, for example leaf worm, can be controlled by spraying across wide swaths. When insects

feed on lower parts of plants and penetration of foliage is required, narrow swaths are more practical. Too wide a swath could also result in an uneven distribution of spray due to variations in wind velocity. Overlapping swaths slightly generally improves coverage.

The choice of pesticide application method is a very important component in the management of pesticides and in the minimisation of risks to the environment resulting from their usage. The means by which applied pesticides may enter the environment are described in the following sections.

4.1.2 Spray drift

Spray drift of pesticides may result from the movement of vapour or particles away from the target area by the action of air flow. Due to the direct input and bioavailability of pesticides carried by spray drift to surface waters, spray drift is often regarded as the worst case scenario in terms of pesticide exposure in aquatic risk assessments (Schulz, 2001). Spray drift can lead to high, yet short-lived, levels of exposure in surface waters (Reichenberger *et al.*, 2007). There are a number of factors which contribute to spray drift which are briefly discussed in this section. In addition to these, it is important to consider physical factors such as the crop canopy and crop distribution, which also affects drift rates (Capri *et al.*, 2005).

The size of the droplets that form upon the formation of a spray as the agricultural chemical passes through a nozzle, is very important in determining the degree of spray drift which may result. Smaller particles have higher diffusion coefficients and are less susceptible to gravitational settling. They therefore remain suspended in the air for longer periods of time than larger droplets, which have higher gravitational deposition rates (refer to Table 4.1). The smaller particles (typically <100 µm) are therefore available to be transported by air turbulence and wind currents to other locations. Droplets of 10 µm, for example, may drift several kilometres at low wind velocities (Hüskes and Levsen, 1997). These small particles cannot be seen with the naked eye, unless they are present in high concentrations resulting in the formation of fog. Smaller droplets also evaporate more quickly due to the presence of a larger surface area to volume ratio.

Table 4.1: Effect of droplet diameter on deposition rates and therefore on drift potential (adapted from Ross and Lembi, 1985)

Droplet diameter, µm	Time to fall 3 m in still air
1 (Fog)	28 h
10 (Fog)	17 min
100 (Mist)	11 s
200 (Fine spray)	4 s
400 (Coarse spray)	2 s
1000 (Coarse spray)	1 s

The velocity at which the droplets exit the nozzle of the applicator impacts on the possibility for spray drift. The speed of the droplets is reduced due to air resistance and gravitational forces. The initial (or exit) velocity of the droplets therefore needs to be sufficiently high enough to allow them to reach the target before drift occurs. In addition, the presence of additives may impact on the degree of spray drift. Density, volatility and viscosity are other important parameters in this regard.

Meteorological factors affecting spray drift include wind velocity and atmospheric stability. The amount of pesticide lost from the target area as well as the distance it moves from the point of application increase with increasing wind speed. Under conditions of high air stability (such as that arising from temperature inversion conditions), slows the settling of small droplets, resulting in them remaining airborne for long periods. Evaporation of spray droplets is enhanced when the relative humidity is low and when the ambient temperature is high, which increases the potential for spray drift due to the consequent decrease in size of the droplets. Evaporation rates are higher for smaller droplets, due to the increased surface area to volume ratio. The evaporation rates are also dependant on the pesticide concerned and its vapour pressure, as well as the formulation used.

There are a number of ways to sample and measure spray drift (Donkersley and Nuyttens, 2011) during pesticide application. Two of the most useful and widely used techniques are based on (1) sedimentation of aerosol onto horizontal surfaces and (2) measurements of airborne concentrations measured at defined downwind locations from the application site by either actively drawing air onto adsorbent surfaces or by passively intercepting the airborne flux with suitable adsorbent materials (Wolters *et al*., 2008). Both of these sets of data are commonly collected during spray drift sampling campaigns. Information on deposition is mainly useful in assessing the risks of pesticides settling into nearby surface water systems (Schulz, 2001) whilst data pertaining to airborne concentrations is important for bystander exposure and inhalation assessments (Butler Ellis *et al*., 2010; De Schampheleire *et al*., 2007; Tupper *et al*., 2012).

The amount of herbicide which was deposited per surface area as a result of spray drift was quantified outside an application area using passive dosimeters by Carlsen *et al.* (2006), which consisted of microscope slides mounted on plastic lids screwed onto wooden sticks providing a sampling height of ~20 cm. In another study, drift traps were employed which consisted of Whatman chromatography paper supported on polyurethane floating tables for water surface measurements, or on wooden stands for land based sampling. The paper was extracted with dichloromethane in an ultrasonic bath, followed by HPLC analysis (Vischetti *et al*., 2008). Capri *et al.* (2005) used a similar paper-based method for assessing deposition of chlorpyrifos in surface water following vineyard applications in northern Italy and paper was also used for a study on Australian cotton (Woods *et al*., 2001). It has been noted that the paper may protect collected pesticides from dissipation processes, such as photodegradation and volatilisation, similar to what occurs in plant leaves and fruit due to the waxy surface layer (Capri *et al*., 2005).

Silicone oil impregnated nylon nets have been employed in the sampling of atmospheric deposition of persistent pollutants, including pesticides (Larsson, 1989) and vertically

oriented pipe cleaners and cotton string have also been employed as collection media (Woods *et al.*, 2001). Straight-sided glass Petri dishes filled with distilled water have been used in another study in order to monitor spray drift deposition (Dabrowski *et al.*, 2005).

Schulz (2001) sampled surface water itself to determine deposition rates from spray drift. This is not an ideal method, however, as dilution by the water would reduce sensitivity. The spray would also form a surface film, where less water soluble pesticides are concerned, thus it may be more appropriate to link the concentration to a surface area, and not to a water volume, as was done in this study. The concentrations of pesticides arising from spray drift using this water sampling method were found to be lower than that predicted by models, which may have been partly due to these effects (Dabrowski and Schulz, 2003).

In some studies, a fluorescent tracer was added to the pesticide spray to determine drift losses, however there is uncertainty with this method due to the possibility of the tracer not following the dispersion of the pesticide (Carlsen, 2006).

An empirical equation has been derived to estimate spray drift rates in field crops (Rautmann cited in Carlsen, 2006):

$$y = 2.7705x^{-0.9787}$$

where y is the ground sediment in % of the application rate and x is the distance in metres from the treated area. With this method, meteorological parameters and formulation and application information are not considered, and it has been found to over-predict spray drift losses (Carlsen, 2006). Other mathematical models which have been developed to estimate pesticide spray drift are reviewed by Gil and Sinfort (2005).

4.1.3 Volatilisation of pesticides

Pesticides may enter the atmosphere upon volatilisation from both soils and plants, and this has been found to be a major dissipation pathway for many pesticides, accounting for up to 90% of the application dose. For many pesticides used in agriculture, volatilisation is a continuous process which begins at the time of application and continues until the residues are depleted (Majewski, 1999). Volatilisation is generally greatest directly after application (with rates ranging from 0.1 g/ha/h to 80 g/ha/h for different compounds), but may continue for a few days to several weeks, and the volatilisation process may display a diurnal cycle due to changes in meteorological conditions (as reviewed by Bedos *et al.*, 2002). The vapour pressure, water solubility and adsorption coefficients (in terms of soil/water partitioning) of pesticides play a key role in determining the rate of volatilisation of the compound after application.

4.1.4 Wet deposition

Precipitation may remove pollutants from the atmosphere by rainout and washout. Rainout occurs when water vapour condenses around pollutant particles during cloud

formation or when gas phase pollutants partition into the rain droplets. Washout, on the other hand, occurs when the falling rain droplets intercept and scavenge pollutant particles or gas phase pollutants below the cloud (Vogel *et al*., 2008). Polar pesticides and particle bound compounds are scavenged with high efficiency from the atmosphere by precipitation (Hüskes and Levsen, 1997). Organochlorine pesticides were first detected in rainfall in the mid-1960s in England, and a wide range of pesticides have since been found in rainfall samples, as wet deposition has been found to contribute more to the total deposition of pesticides than dry deposition (Dubus *et al.*, 2000). Persistent chlorinated pesticides, for example, were found in precipitation from the Lake Malawi area of Southern Africa (Karlsson *et al.*, 2000). A study found that the mass of pesticides deposited by rainfall was <2% of the total applied dose in the areas tested (Vogel *et al.*, 2008).

The timing and proximity of the rainfall event to pesticide application areas strongly influences the occurrence of pesticides in rainfall. Some pesticides are detected up to a few months after the local spraying season as a result of volatilisation from treated soils and plants and due to their long residence time in the atmosphere (Dubus *et al*., 2000). The effect of simulated rain on deposits of pesticides on cotton was investigated in South Africa (Pick *et al.*, 1984), where it was found that 2 to 5 mm of simulated rainfall one hour after application of the pesticide washed off >50% of the original deposit.

4.1.5 Modelling

Monitoring environmental exposure during and after spray operations can be difficult because of the variation in environmental conditions. Air monitoring of pesticide spray drift, which involves sampling, extraction, and chemical analysis, for the large geographical areas involved can be an exorbitantly expensive and time-consuming exercise. The long sampling period, in the order of hours for most methods, in relation to the relatively short spray event also does not allow for an understanding of the evolution of any spray event (Tsai *et al*., 2005).

Evaluating all possible interactions of the numerous application variables can be complex, therefore a computer model is the most practical way to conduct spray drift risk assessments (SDTF, 1997). Models, however, can give different results from real field data due to factors which may be difficult to account for mathematically and computationally. For example, discrepancies may be due to the pesticide's interactions with the surroundings as well as temporal and spacial variations in the environmental conditions (Gil and Sinfort, 2005), which are difficult to model. It is therefore important to conduct monitoring studies in order provide the necessary information for the development, calibration and validation of emission, atmospheric transport and deposition models (van Dijk and Guicherit, 1999) before they can be used with confidence.

In this study the AGricultural DISPersal (AGDISP) model was chosen for evaluation, which is used to simulate pesticide spray drift during application. It was originally developed to simulate only aerial application scenarios (Bilanin *et al*., 1989). It is based on equations of motion for droplet trajectories as they are being released from spray

nozzles. Additionally, for aerial applications the model includes simplified equations that describe the effects of the aircraft-wake and ambient turbulence (Huang *et al*., 2010; Teske *et al*., 2003).

Through comparative monitoring studies, the model has undergone several modifications including the addition of a single evaporation constant to account for evaporation of the active, additive, and carrier components of a spray mix (Escoffier and Scire, 2013). Another modification was the addition of a feature for simulating ground application with boom sprayers (Teske *et al*., 2009), which is a common practice. Most of the validation studies of the ground feature of the model, however, were not conducted using the active ingredient, i.e. the pesticide, but rather by using a fluorescent tracer compound (Connell *et al*., 2012; Fritz *et al*., 2011; Teske *et al*., 2004; Teske *et al*., 2009) or metal salt (Woodward *et al*., 2008; Zabkiewicz *et al*., 2009) added to the spray solution for this purpose.

As discussed in Section 1.2, spray drift is dependent on a number of parameters which include: (i) the application technique being used (Foqué *et al*., 2014; Huang and Thomson, 2011), (ii) the canopy type and height (Hoffmann *et al*., 2007), (iii) meteorological conditions (Bird *et al*., 1996; Miller *et al*., 2011) and (iv) the physiochemical properties of the spray material being applied (Fritz *et al*., 2010; Hewitt *et al*., 2009; Hilz and Vermeer, 2013). It is therefore important to correctly measure (or estimate) these parameters when performing spray drift simulations.

4.1.6 Target pesticide

Spray drift field studies were conducted in this study during the application of a widely used chlorinated triazine herbicide, namely atrazine, used in sorghum production for controlling both pre- and post-emergent broadleaf weeds. Table 4.2 shows the physical and chemical properties of this herbicide and the annual usage of atrazine in South Africa on sorghum and maize is given in Table 4.3. There were a number of factors which determined the choice of this pesticide for study, which are elaborated on here. Muir *et al*. (2004) showed by means of both empirical and modelling approaches that atrazine is among the pesticides that are capable of regional atmospheric transport, thus making it an important pesticide to study. A recent study by Dabrowski *et al*. (2014) dealing with the prioritization of pesticides that are used in South Africa, featured atrazine as the highest ranked pesticide amongst 25 priority pesticides. The ranking and prioritization approach was based on a weighted hazard potential (WHP) – a prioritization index which is directly proportional to the pesticide's toxicity potential (TP) and total quantity used. Owing in part to its wide use in maize production, this herbicide has been detected in South African freshwater systems in a number of studies (Dabrowski *et al*., 2013; Du Preez *et al*., 2005; Pick *et al*., 1992). The use of atrazine as a spray drift tracer in this study was also motivated by its ease of analysis, which does not require a derivatization step, as this can incur analyte losses and elevated analytical uncertainties. Atrazine can be quantitatively analysed by either gas chromatography with electron capture detection (GC-ECD) or with mass spectrometry detection (GC-MS).

Table 4.2: Physical and chemical properties of atrazine (ATSDR, 2003)

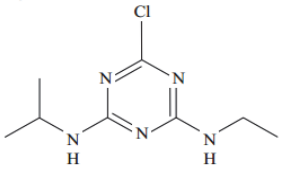
Property	Information
IUPAC name	6-chloro-4-N-ethyl-2-N-propan-2-yl-1,3,5-triazine-2,4-diamine.
Molecular weight	215.69 g/mol
Chemical structure	
Boiling point	279°C
Melting point	173-175°C
Density (at 22°C)	1.23 g/cm^3
Solubility:	
in water (at 22°C)	34.7 mg/L
in acetone (at 25°C)	31 g/L
in n-hexane (at 25°C)	0.11 g/L
Vapour pressure (at 25°C)	2.89×10^{-7} mmHg
Henry's Law constant (at 25°C)	2.96×10^{-9} atm-m^3/mol
Hydrolysis rate constant (at 25°C)	2.735×10^{-11} cm^3/molecule-second

Table 4.3: Use of atrazine in South Africa for the year 2009 (see Volume 2 of this report)

Crop	Crop area (10^3 ha)	Area treated (10^3 ha)	Dose rate (kg or L/ha)	Volume (10^3 kg or L)
Sorghum	87.00	20.42	2.106	43.015
Maize	2750.00	915.89	0.936	890.981

Atrazine deposition from the atmosphere to surface water can have harmful effects on aquatic life, and it is known to have endocrine disrupting activity with respect to aquatic animals (Giusi *et al*., 2006; Hayes *et al*., 2010; Hrouzková and Matisová, 2012). Alarmingly, some studies have shown it to be an endocrine disruptor in human cells (Fan *et al*., 2007; Sanderson *et al*., 2000). Atrazine can be quite persistent in water and a study by Thurman and Cromwell (2000) in Lake Superior in Canada, suggested that the half-life of atrazine in lakes was over 10 years. Atrazine can undergo transformations in the environment to de-alkylated, hydroxylated, and chlorinated products (Qiao *et al*., 1996). It is also persistent in soil where it has a half-life of between 77-101 days in surface soil and over 900 days in subsurface soil (Blume *et al*., 2004).

In the atmosphere, atrazine is fairly stable and has been shown to be capable of undergoing regional atmospheric transport of over 1 000 km (Muir *et al*., 2004). The degradation pathway of atrazine in the atmosphere is through •OH radical attack. If this

attack occurs with a rate constant of 1.4×10-11 cm^3/s, and if the •OH concentration is between 5-10 ×10-5 cm^3, then the average lifetime of atrazine is approximately 1 day. However, it has been shown that if the mean wind speeds are 3-5 m/s, then atrazine can travel 250-500 km within a day before any degradation can occur (De Rossi, 2010). Thus the atmosphere serves as a potential exposure route especially for animals through inhalation.

The stability of atrazine in the atmosphere makes it suitable for use as a spray drift tracer during application. A study by Ravier *et al*. (2005) successfully used atrazine to monitor atmospheric spray drift up to 134 m downwind. Atrazine can be easily trapped onto sampling media such as filter paper or polyurethane foam (PUF) and, as previously mentioned, analytical techniques such as GC-MS or GC-NPD may be used for quantitation.

4.2 AIM AND OBJECTIVES

The aim of this part of the project was to validate an air dispersion model (AGDISP) which could potentially be used to predict spray drift for the human exposure risk assessment.

The specific objectives were therefore to:

1. Monitor pesticide spray drift during pesticide application at a large scale agricultural farm. This required that a method to sample and quantify the spray drift of the active pesticide be developed and validated.
2. Use the data collected during the sampling campaign as input data to the AGricultural DISPersal (AGDISP) model and then to compare the model output with the monitoring data for model validation purposes. In addition, necessary recommendations in terms of model uncertainties for using the model under local conditions were to be determined.

4.3 METHODOLOGY

4.3.1 Study Area

Spray drift monitoring was done during spraying of weeds in a sorghum field in Standerton, South Africa on 28 January 2014 under calm meteorological conditions. The field concerned was 7.6 ha which was surrounded by the farm house and some grazing land for the farm animals. A total of approximately 1000 hectares of sorghum are grown on this farm. The spray solution was applied using a John Deere 4720 ‘Hi-Boy’ ground sprayer equipped with 56 TwinJet® hydraulic nozzles (TeeJet® Technologies) which were uniformly distributed at 0.5 m intervals on a 32 m boom (Figure 4.1). The crop was 0.9 m high and application was done at 0.4 m above the crop. The herbicide mix contained atrazine, terbutylazine, and other triazines as active substances.

Figure 4.1: (a) The ground sprayer with 56 nozzles along a 32 m boom used to apply the atrazine mix in Standerton (b) Visible spray drift away from the target crop during application.

4.3.2 Determination of sampling positions

A good estimate of the wind direction was determined using a portable RM Young instrument while setting up the samplers, which deviated only 29° from the average wind direction that was measured later during the sampling period. The downwind setup of the samplers was similar to that of Caldwell (2006) and is shown in Figure 4.2. The distances were determined using a Nikon LASER range finder (model 1200S).

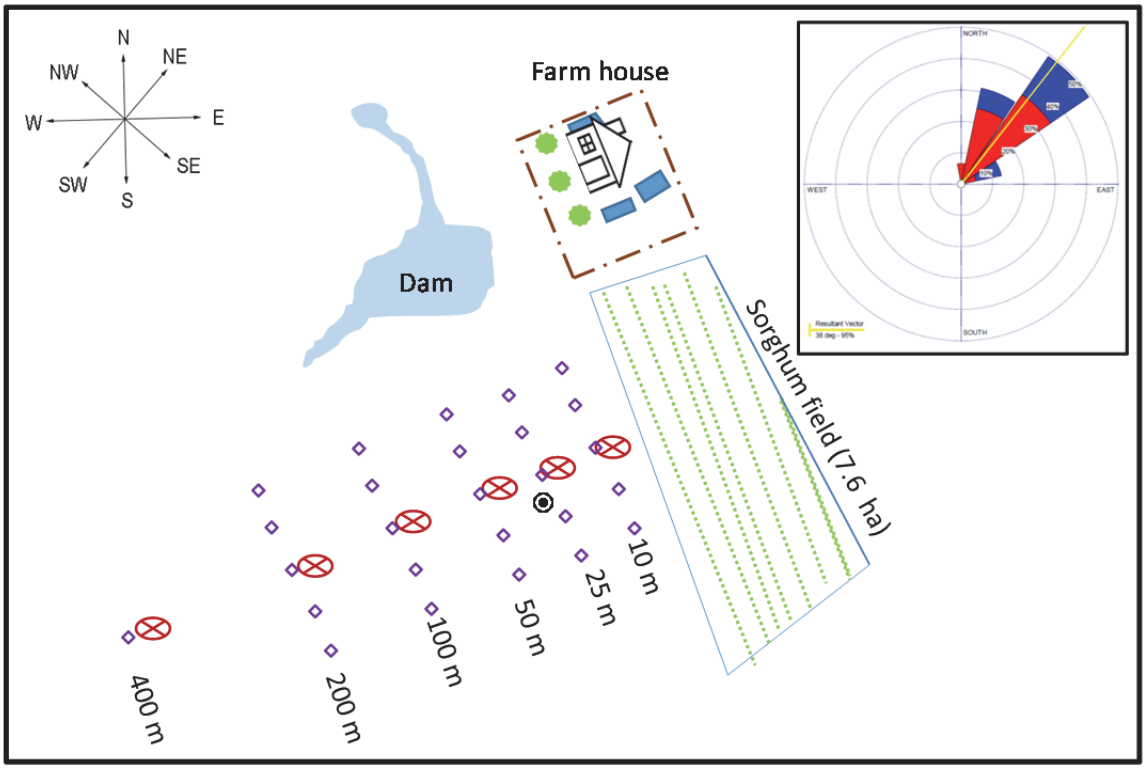


Figure 4.2: Schematic diagram showing sampling points downwind from a pesticide application area, the sorghum field. Airborne drift samplers ⊗ were located downwind as shown. The deposition collectors ◊ were positioned 5 m apart at each sampling location. A weather station ⊙ was set up at the site as indicated. The inserted wind rose shows average wind direction during the 1 hour sampling period (adapted from a Google Earth image of the area).

4.3.3 Deposition sampling

The samplers used for deposition sampling were distributed as shown in Figure 4.3. Each of these consisted of a 27 × 15 cm piece of Whatman™ chromatography paper which was used without prior treatment. This material has been used in previous studies for spray drift deposition monitoring (Miller *et al.*, 2011; Woods *et al.*, 2001). Each piece was placed on an aluminium foil covered support stand which was mounted 20 cm above ground and the paper was held in place with paper clamps (Figure 4.3). Deposition was collected during a 1 hour period after which the chromatography paper was carefully removed from the stand, covered with aluminium foil, and stored in sealed plastic bags. These samplers were then transferred to the laboratory where they were stored at 3°C prior to analysis. Non-exposed paper was used as a blank.

Figure 4.3: Chromatography paper (27 × 15 cm) mounted 20 cm above the ground and held in place with clamps on an aluminium surface for spray drift deposition sampling.

4.3.4 Active airborne sampling

Polyurethane foam (PUF) plugs (6 cm diameter, 4 cm long) were used to trap atrazine during active air sampling, as done previously by Gish *et al.* (1995). These were prepared by loading two pre-cleaned PUF plugs, in series, inside an aluminium cartridge. Four of these cartridges were connected to a generator powered vacuum pump through a manifold. The cartridges were positioned at four different vertical distances (0.5, 1, 1.5, and 2 m above ground) by means of a mast as shown in Figure 4.4. Air was drawn through each PUF cartridge at an average flow rate of 348 L/min for 1 hour. The flow rate of each pump was calibrated using a Planton GAP meter. After sampling, each cartridge was sealed in aluminium foil and taken to the laboratory for analysis.

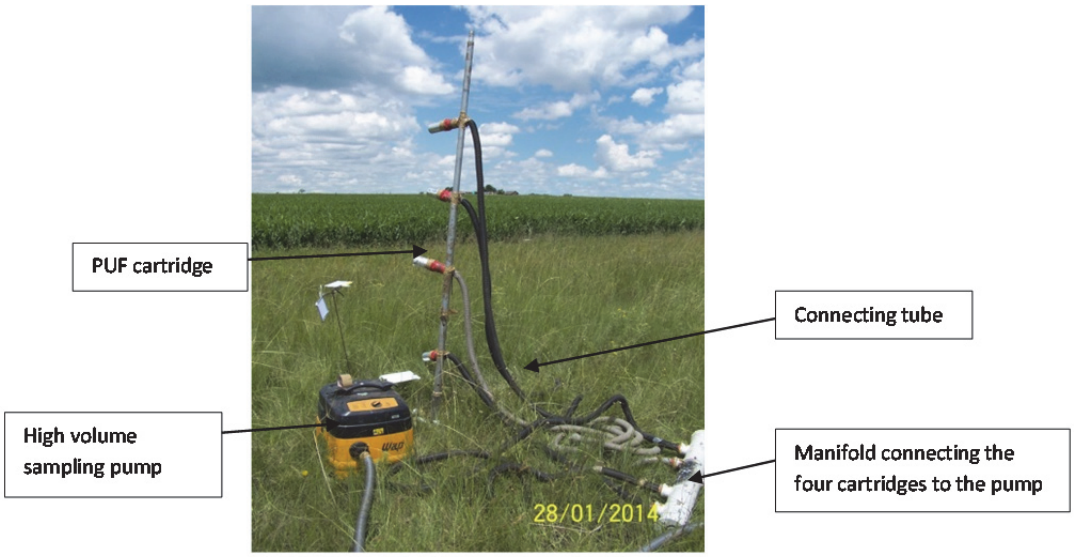


Figure 4.4: PUF active samplers used to draw air at over 220 L/min. 4 PUF cartridges were mounted vertically on a mast at four heights (0.5, 1, 1.5, and 2 m) and connected to a pump through a manifold.

4.3.5 Passive Airborne Sampling

Wool traps made of a 21 x 21 cm aluminium frame into which 3 mm grooves at 5 mm intervals had been machined were used where wool had been wound in between the groves in a zigzag fashion (Figure 4.5 a-b). The wool was pre-cleaned by soaking it in a 50:50 (v/v) mixture of acetone and hexane, after which it was allowed to air dry under ambient conditions. The traps were held with clamps onto a metal rod 90 cm above the ground (Figure 4.5c) and they were clamped vertically and horizontally to intercept airborne flux and to collect deposition, respectively. Five of these traps were placed 10 m apart at each of the six downwind locations as shown in Figure 4.2. After the sampling period, each wool trap was wrapped in aluminium foil and placed in a sealed plastic bag. They were then taken to the laboratory where they were stored in a refrigerator. Due to budgetary constraints, these samples were not analysed, but were archived for future studies.

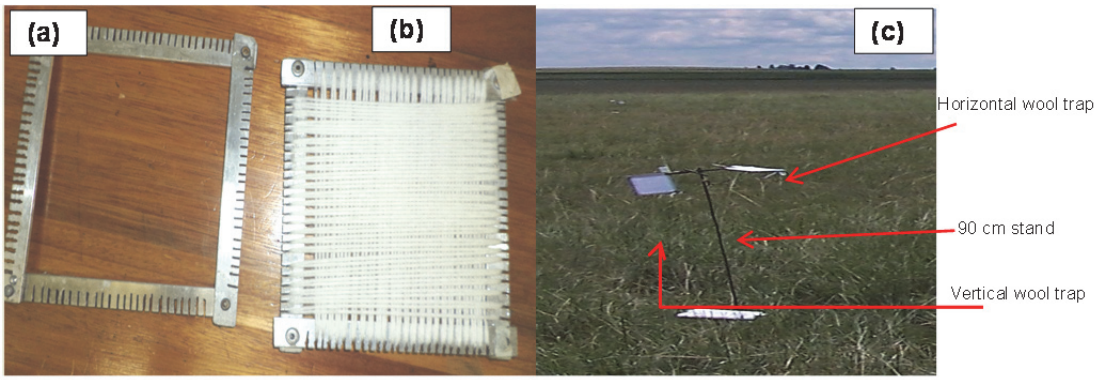


Figure 4.5: (a) Aluminium frame with 3 mm grooves at 5 mm intervals. (b) Wool trap made by winding wool into the groves. (c) Field setup of the wool traps on a 90 cm high stand.

4.3.6 Extraction and analysis of samples

4.3.6.1 PUF Samples

PUF samples were extracted by means of a plunger based solvent extraction technique previously validated by the Agricultural Research Council (van der Walt, 2000). The method was however slightly modified for this particular application where an acetone:hexane (1:3) mixture was used as an extraction solvent, since other studies had successfully used it for atrazine extraction (Majewski *et al*., 1998). Both the solvents were high purity pesticide grade Burdick and Jackson (Anatech Pty Ltd). Each PUF plug was extracted with 25 mL of the solvent mix by repeatedly squeezing it with a plunger (Figure 4.6) 10 times and collecting the extract. This was repeated 5 times, adding 25 mL of fresh solvent each time. All the extracts from the same PUF cartridge were combined and filtered through filter paper (Lasec SA Pty Ltd). Pre-concentration of the combined extracts was then performed using a rotory evaporator at 40°C, and the extract was made up to a final volume of 5 mL.

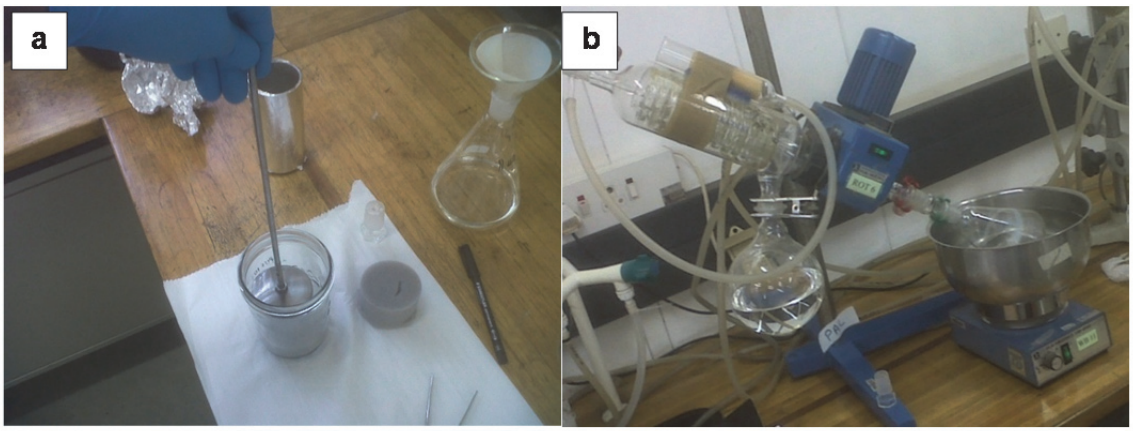


Figure 4.6: The extraction of PUF plug samples for atrazine determination. (a) Plunger extraction with extraction solvent to extract the trapped pesticides. (b) Pre-concentration of extracts by rotory evaporation

Analysis and quantification of atrazine was carried out using an Agilent 6870N gas chromatograph equipped with a nitrogen phosphorous detector (GC-NPD) (Agilent Technologies, Inc.). 1 µL of sample was injected in splitless mode and the carrier gas was nitrogen at a flow rate of 1.0 mL/min. A DB-5MS fused silica column (30 m × 0.25 mm ID) with a 0.25 µm film thickness (Agilent Technologies JandW Scientific) was used. The temperature programme started at 60°C (held for 1 min), then was raised at 25°C/min to 210°C (held for 4 min), and was finally taken to 250°C at 30°C/min. The detector temperature was 310°C and the detector gas flow rates were 4 mL/min (hydrogen) and 60 mL/min (air) and the makeup gas (nitrogen) was 22 mL/min. Chromatograms were integrated using OpenLAB Chromatography Data System (CDS) ChemStation Edition software (Agilent Technologies). All the gases for the instrument (Synthetic Air, Nitrogen 5.0, and Hydrogen 5.0) were of high purity and were supplied by Air Products.

4.3.6.2 Quality assurance/quality control

An atrazine standard (PESTANAL®, 98.8% purity) from Sigma-Aldrich® was used for external calibration of the instrument and for surrogate spikes. A good linear fit was found for the calibration standards (R^2 = 0.9956) and the limit of detection (LOD) was found to be 8.66 pg and the limit of quantification (LOQ) was 25.99 pg. The method showed good repeatability (3.3% RSD, n = 6) and the mean recovery of PUF cartridges spiked at three different concentrations was 94.5% (3.0% RSD). Blank solvent was periodically injected between sample runs to ensure no analyte carryover occurred. Sensitivity was constantly checked throughout the analysis by injecting calibration standards. Field banks were also taken to account for atrazine residues that might have been in the atmosphere before spraying.

4.3.6.3 Deposition Samples

A 5 × 2 cm strip was cut from the middle of each of the chromatography paper sampling sheets and was shaken with 5 mL hexane:acetone (3:1) mixture in an ultrasonic bath for 30 min. These extracts were stored in a refrigerator with the chromatography paper inside the vials until analysis. Semi-quantitative analysis of the extracts was done with a Perkin Elmer AxION® 2 time-of-flight mass spectrometer integrated with the AxION® Direct Sample Analysis™ inlet (DSA-TOFMS). Internal calibration of the instrument was performed using m/z 121.05087 and 622.02896 as lock mass ions (Agilent APCI/APPI tuning mix). The rest of the operating conditions are shown in Table 4.4. A 5 µL portion of the extracted sample was injected onto the mesh grid (sample holder) for introduction into the DSA-TOFMS system. The instrument uses a nitrogen plasma to desorb and ionise the sample from the mesh grid and the stream of ions thus produced enters the time-of-flight mass spectrometer. The analysis of each sample was extremely fast and took 25 seconds per sample. A pesticide library (AxION Solo™) was used for identification of atrazine peaks.

In order to obtain quantitative information from the instrument, external calibration was done using atrazine standards (from 10-1090 pg/µL) where 5 µL of each concentration

was analysed under the same conditions as the samples. The instrument showed good linearity (R^2 = 0.9934) and this allowed for semi-quantitation of the samples. In addition, the same sample extracts were analysed using the GC-NPD instrument using the same method used for the airborne PUF samples and the two techniques were compared.

Table 4.4: Operating conditions of the Perkin Elmer AxION® DSA-TOFMS instrument used to analyse pesticide spray drift deposition paper samples.

MS Conditions		DSA parameters	
Ionization source	AxION® DSA™	DSA heater temperature	250°C
Ionization mode	Positive	Auxiliary gas	80 psi
Spectral acquisition rate	5 spectra/sec	Drying gas flow rate	3 L/min
Capillary exit voltage	100 V	Drying gas temperature	25°C
Detector voltage	8 000 V	Corona needle voltage	2 000 V

4.3.7 Spray Drift Modelling

The AGricultural DISPersal (AGDISP) v8.27 programme was used to model the spray drift. It uses both Lagragian and Gaussian-type equations to calculate the droplet trajectories as they are released from the nozzles. The reader is referred to (Teske *et al*., 2009; Teske *et al*., 2003) for a detailed discussion on the mathematics and algorithms utilised by the model. The predictions of pesticide deposition and downwind drift generated by the model are dependent on a set of parameters which must be consistent with those under which the pesticide application was carried out. In brief, this set of inputs must represent the equipment conditions, the nozzles and the drop size distribution (DSD) they create, spray material properties, surface features (topography), and ambient meteorology. Such information was therefore collected in this study during the sampling campaign and is presented in Table 4.5.

Meteorological data was continuously monitored throughout the application and sampling period using an RM Young set of instruments mounted on a mast which was positioned ~25 m downwind. Data was measured every second and was logged as 1 min averages of wind speed and direction (RM Young model 0513 Wind Monitor-RE, Young Co.). Temperature and relative humidity were measured with an RM Young Multi-plate radiation shield (model 41003), while solar radiation was monitored with an RM Young pyranometer (model PY51486). All the meteorological data was logged by means of a datalogger (model CR215, Campbell Scientific, Inc.) and later downloaded to a computer with LoggerNet software (version 7.0). The data was then averaged over the 60 min sampling period corresponding to the 10 min pesticide application time and 50 min post-application sampling time, similar to previous spray drift studies (Fritz *et al*., 2011). The meteorological data is shown in Table 4.6.

To determine the droplet size distribution produced by the sprayer, the magnesium oxide method initially described by May (1950) was used. Magnesium oxide-coated slides were clamped on rods 1 m above ground which were placed along the edge of the sorghum field to capture spray droplets during application. The droplets made impressions on the magnesium oxide slides. Images of these impressions were taken using a Zeiss Stemi SR microscope equipped with a Schott KL 1500-Z light source and AxioVision (version 3.0.6.38) software at 20x magnification. Image analysis using

Droplet Retrograde software (2004 version) was performed to determine the DSD. The droplet size distribution that was generated was then input to the AGDISP model as a "User-defined" input for computation.

Default values for a number of parameters were not changed and are included in Table A2 of Appendix 2:. Since the spray material was predominantly water, the evaporation rate of water (84.76 µm2/°C/s) was used as an estimate of the evaporation rate of the atrazine spray material (Holterman, 2003; Riley et al., 1995; Teske et al., 1998). This is supported by the studies which showed that agrichemicals in water-based carriers will behave more or less like water in terms of evaporation (Hewitt et al., 2002b). An example of all the model input parameters required is shown in Table A2.

Table 4.5: Input parameters for AGDISP used for modelling spray drift

Parameter	**Input**	**Parameter**	**Input**
Application method	Ground sprayer	Wind speed (m/s)	5.17
Boom pressure (bar)	4.8	Wind direction (°)	-90
Nozzle type	Flat fan	Temperature (°C)	26.57
Number of Nozzles	56	Relative Humidity (%)	47.49
Nozzle inter distances (cm)	50	Spray material evaporates	Yes
DSD	User-defined	Spray volume rate (L/ha)	150
Release height (m)	1.4	Active Fraction	0.0113
Spray lines	8	Active fraction of tank mix	0.0113
Swath width (m)	32	Evaporation rate (μm^2 /°C/s)	84.76
Swath displacement (m)	0	Canopy Height (m)	0.9

Additionally, the model sensitivity to various input parameters was investigated by varying one parameter while keeping the others constant. The parameters that were investigated are shown in Table 4.6.

Table 4.6: AGDISP sensitivity test matrix

Parameter	**Input values**		
	Min/Trial 1	**Average/Trial 2**	**Max/Trial 3**
Temperature (°C)	25.98	26.58	27.08
Relative humidity (%)	40.57	47.49	55.30
Wind speed (m/s)	3.35	5.17	7.27
Evaporation Rate (μm^2/°C/s)	74.79	84.79	94.79
Droplet size distribution – VMD (µm)	534.34	600.64	693.27
Application rate (L/ha)	100.00	150.00	200.00

The meteorological parameters were varied according to maximum, minimum, and average values that were obtained during the sampling period while the other parameters were varied to accommodate possible variations in these values.

4.3.7.1 Scenario Modelling Inputs

Scenario modelling for three different locations of relevance to this study, namely Bothaville, Letsitele, and Komatipoort, was done to compare the outputs of the model for different application conditions and for different pesticides for use in the human risk assessment component of this study. Input parameters for each pesticide expected to be used in each area based on the crops grown there, included the typical or recommended application rates provided by the pesticide manufacturers. The input for each pesticide modelling scenario consisted of the spray volume rate, the percentage of active ingredient, the height of crop during application, and the boom height (Table 4.7). The input data for the Komatipoort modelling scenario was obtained from a local sugar cane farmer in Komatipoort who normally applies pesticides with a small ground sprayer equipped with 12 nozzles along a 5 m boom. Typical (average) meteorological conditions for the three areas were used based on data obtained from the South African Weather Service, and the rest of the parameters were kept the same as those for the Standerton study. The model's inbuilt 'fine' droplet size distribution was assumed in all cases to simulate a worst case scenario.

Table 4.7: Model inputs used for pesticide application scenario modelling for Bothaville, Letsitele and Komatipoort.

Area	Pesticide	Spray volume rate (L/ha)	Active ingredient in tank mix (%)	Crop height (m)	Boom height (m)
Bothaville	Atrazine	200	0.3	0.96	1.5
	Alachlor	200	0.984	0#	0.5
	Simazine	200	0.375	0#	1.5
	Terbutylazine	200	0.3	0.5	1.5
	Metolachlor	200	0.0771	0#	0.5
Letsitele	Propiconazole	2000	0.005	2.5	2.9
	Imazalil	2000	0.05	2.5	2.9
	Thiabendazole	2000	0.15	2.5	2.9
	Imidacloprid	2000	*	*	*
Komatipoort	Diuron	36.8	0.5	0.6	0.9
	Paraquat	36.8	0.5	0.6	0.9
	Glyphosate	36.8	0.5	0.6	0.9
	MSMA	36.8	0.5	0.6	0.9
	Metribuzin	36.8	0.5	0.6	0.9

**Applied directly to the soil and not sprayed*
 # Pre-emergent application

4.4 RESULTS AND DISCUSSION

4.4.1 Airborne Drift

An example of a chromatogram obtained for a PUF extract is shown in Figure 4.7. The GC-NPD method showed good resolution of the atrazine peak (at 8.26 min) from the other closely eluting triazines especially terbuthylazine. Although the detector gave a relatively high baseline, the atrazine peaks were well above the detection limits for all samples, thus peak-integration, and hence quantitation, was possible. The other peaks in the chromatogram could be due to other impurities in the air samples, additives in the spray mix, or even impurities in the PUF adsorbents that were used. These peaks were, however, not further investigated because they did not interfere with the atrazine peak of interest.

Figure 4.8 shows the air concentrations of atrazine determined from the PUF samples relative to the downwind distance from the edge of the field and at different vertical sampling heights, which were calculated based air sampling flow rates. The percentage recoveries were not used in calculating these results as is common practice in environmental monitoring studies where recoveries are good (Ratola *et al*., 2014; Thompson *et al*., 1999). The experimental PUF results show a decrease in airborne atrazine concentrations with downwind distance – a trend that was observed for all four sampling heights. Closer to the field (10 and 25 m) there was a greater variation in atrazine concentration with vertical height compared to samples from further downwind. This could be because the effect of the moving tractor is much more significant near field compared to far-field distances. Also, as the spray cloud drifts away, it disperses and mixes with the atmosphere to form an expanded plume of almost homogenous concentration. It can also be noted that that near-field (10 and 25 m downwind) the atrazine levels were slightly lower at 0.5 m compared to 1 m above ground. This could be because the material was released around 1.4 m and therefore a majority of the droplets had still not settled closer to the ground. The lowest concentration (0.138 pg/L) was detected at 400 m downwind at 2 m vertical height.

Single ANOVA treatment of the data showed that there was no significant difference in concentrations of samples that were taken at different heights ($p > 0.05$). However, visual comparison shows that samples taken at 2 m height have slightly lower concentrations compared to the other heights. This could be attributed to both deposition of the pesticide to the ground as well as diffusion processes.

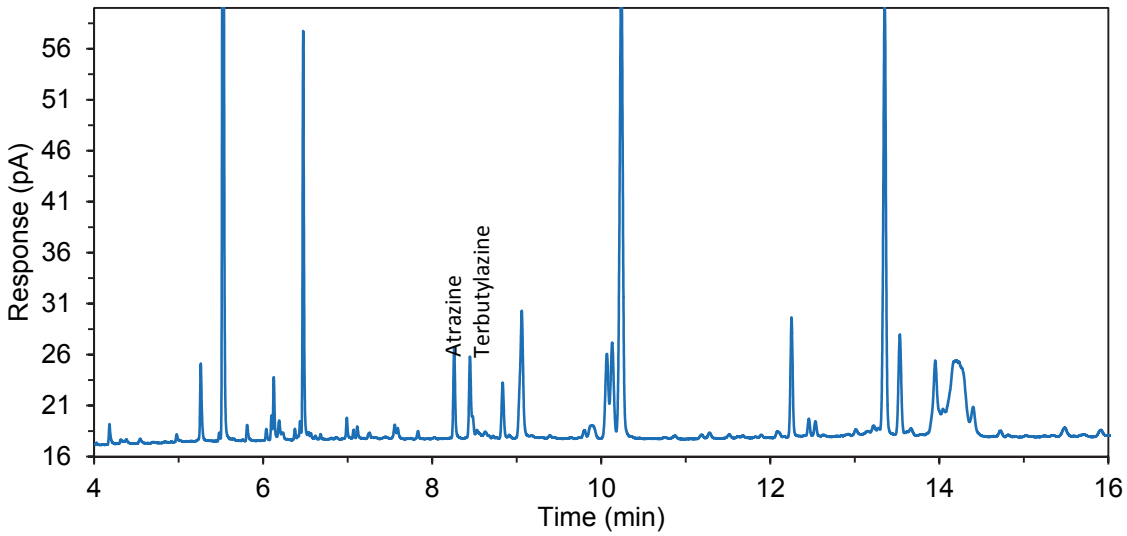


Figure 4.7: Chromatogram of the PUF extract from 400 m downwind and 2 m vertical height. It shows atrazine and terbutylazine peaks at 8.267 and 8.457 min respectively. The other peaks did not interfere with the peak of interest and therefore were not investigated.

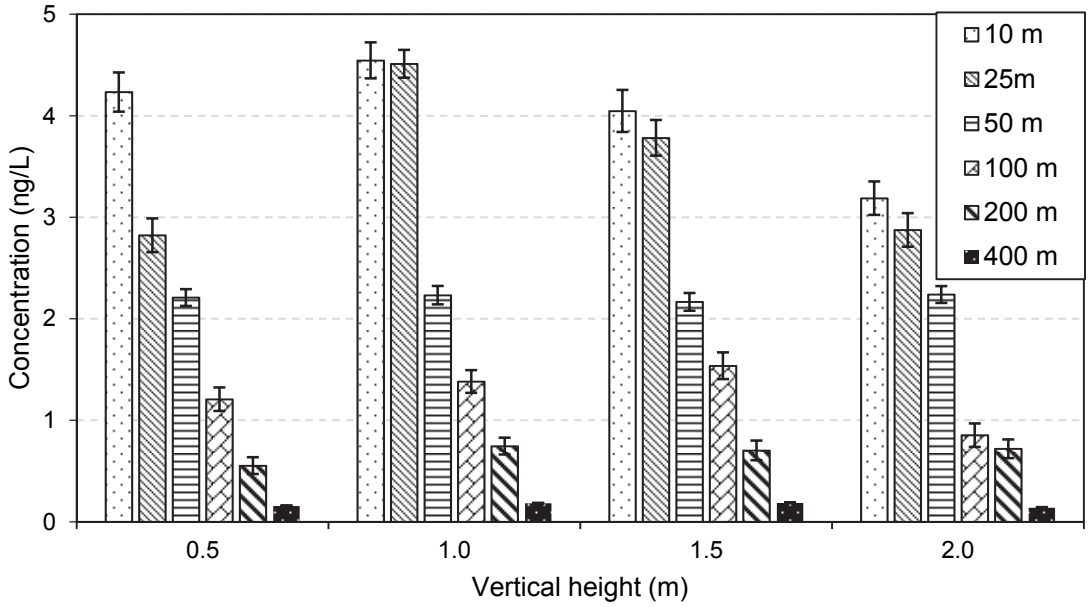


Figure 4.8: Measured atrazine concentrations in PUF samples expressed per volume of air sampled over one hour at the six downwind locations and at the four vertical heights.

4.4.2 Comparison between model and experimental airborne drift

A comparison of modelled versus experimental airborne concentrations at the different vertical heights is shown in Figure 4.9. The model results showed the expected general decrease in concentration with downwind distance, a trend that was also observed for the experimental results. However, the model results showed a marked decrease in concentration with vertical height from 1 to 2 m while the experimental results showed only a slight decrease. This is could be a consequence of the actual versus predicted rates of volatilization and dispersion of the spray plume. Linear regression results

showed that for all the sampling heights there was a good correlation between experimental and model generated results ($R^2 > 0.79$).

The correlation between experimental and model results was found to be very dependent on the average droplet size (Figure 4.9). From the analysis of three different MgO slides, three different droplet size distributions were obtained. The best correlation between experimental and modelled results was observed when the droplet distribution with an average size of 166.484 μm was used (Figure 4.9, VMD693). When simulations were done with smaller droplet sizes (145.253 μm), the model over-predicted the concentration by up to 3 times near-field but predicted the concentration further downwind better (Figure 4.9, VMD534). Using 207.957 μm diameter droplets (Figure 4.9, VMD600) gave a reasonable correlation to the experimental results although the model under predicted concentrations closer to the field. These results show the importance and sensitivity of the model output to the droplet size where slight changes can have a significant impact on the model output.

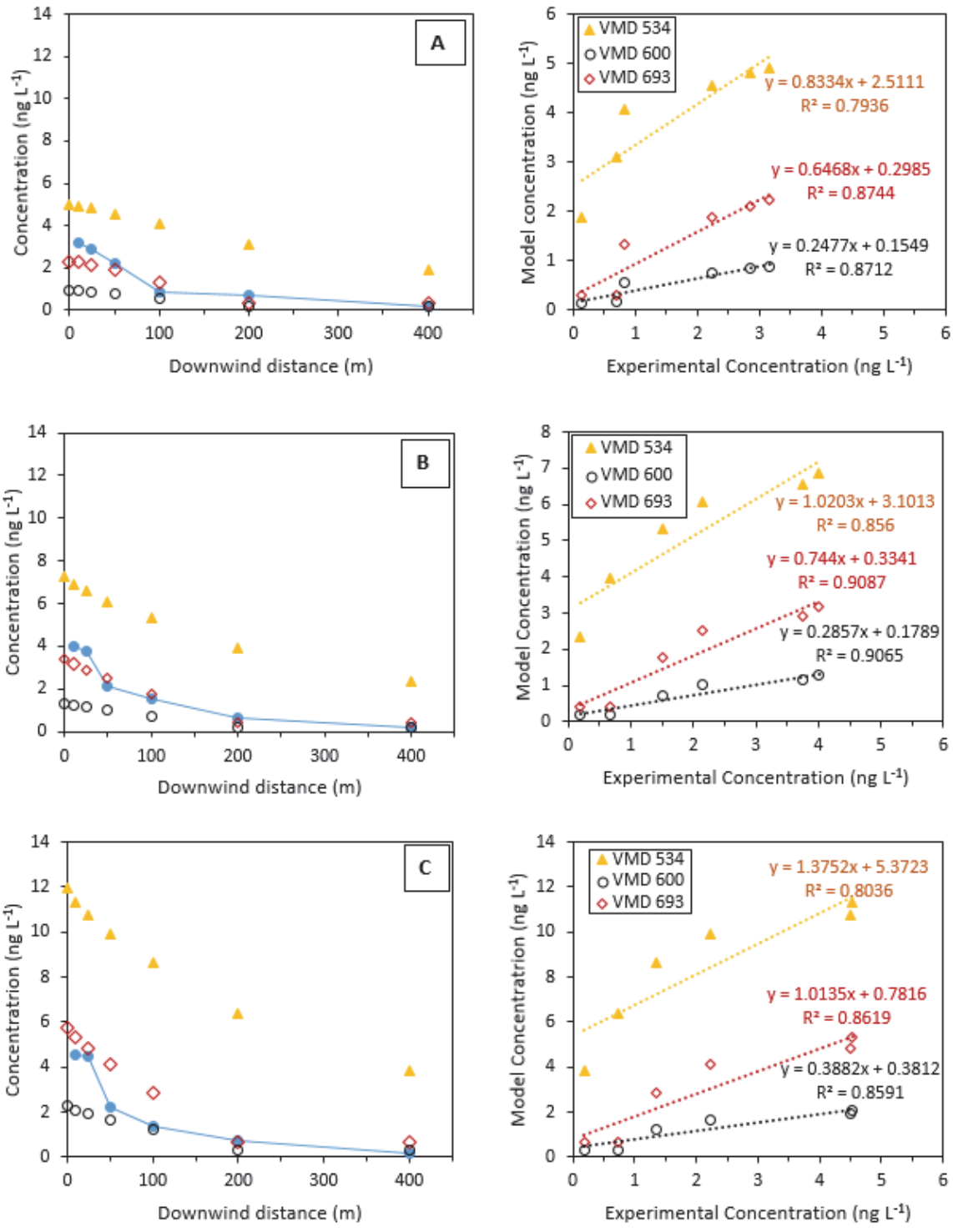


Figure 4.9: Left: Comparison of model • vs experimental results at three different vertical heights (a) 2 m (b) 1.5 m (c) 1.0 m. Three model runs were made using different droplet distributions ×VMD600 (207.957 µm diameter) ◇VMD693 (166.484 µm diameter) ▲VMD534 (145.253 µm diameter). Right: Correlation between experimental and model results for each of the droplet sizes.

4.4.3 Pesticide Deposition

Deposition results predicted by the AGDISP model are shown in Figure 4.10 and the experimentally measured deposition rates of the field samples are shown in Figure 4.11. For smaller droplet sizes the model predicted higher downwind deposition rates compared to larger droplets, as expected because larger droplets are less susceptible to drift and tend to settle within the sprayed area while the lighter droplets are more prone to drift and therefore deposit downwind.

Compared to the experimental results, the model under-predicted the deposition by up to one order of magnitude compared to the GC-NPD results and even more compared to the DSA-TOFMS results close to the field. This is in contrast to a study by Woodward *et al*. (2008) which also attempted to validate the ground application feature of AGDISP. They found that the model over-predicted the downwind deposition by a factor of 3.5-100. A very similar study by Zabkiewicz *et al*. (2009) also reported an over-prediction of downwind deposition by the model. The difference between the results and could be due to the different field monitoring approaches. The other studies used metal ions as tracers while this study used the actual active ingredient in the spray solution to monitor the pesticide drift and deposition rates.

There was also a discrepancy within the experimental results of this study. GC-NPD analysis gave lower concentrations compared to those from DSA-TOFMS, especially near to and far from the field, with a good correlation between methods being evident for the 50 and 100 m distances from the field. The samples were first analysed on the DSA-TOFMS instrument before they were taken for analysis by GC-NPD. It is possible that a portion of the atrazine in the samples was lost due to adsorption onto the walls of the storing glassware, hence the lower concentrations determined by GC-NPD. Another reason could be purely due to the differences in the instrumental techniques themselves.

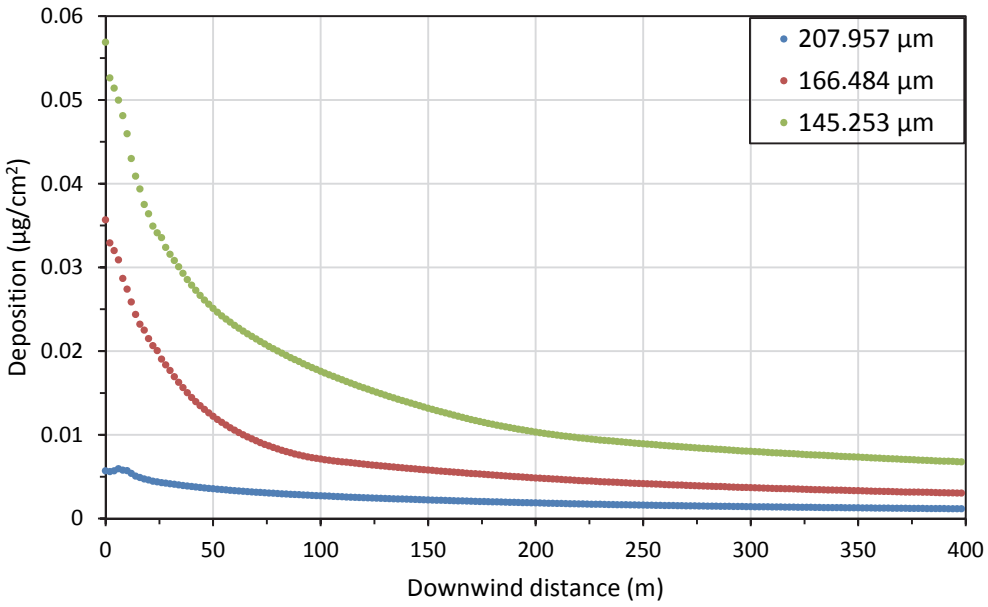


Figure 4.10: Downwind deposition rates predicted by the AGDISP model using three different droplet size distribution sets.

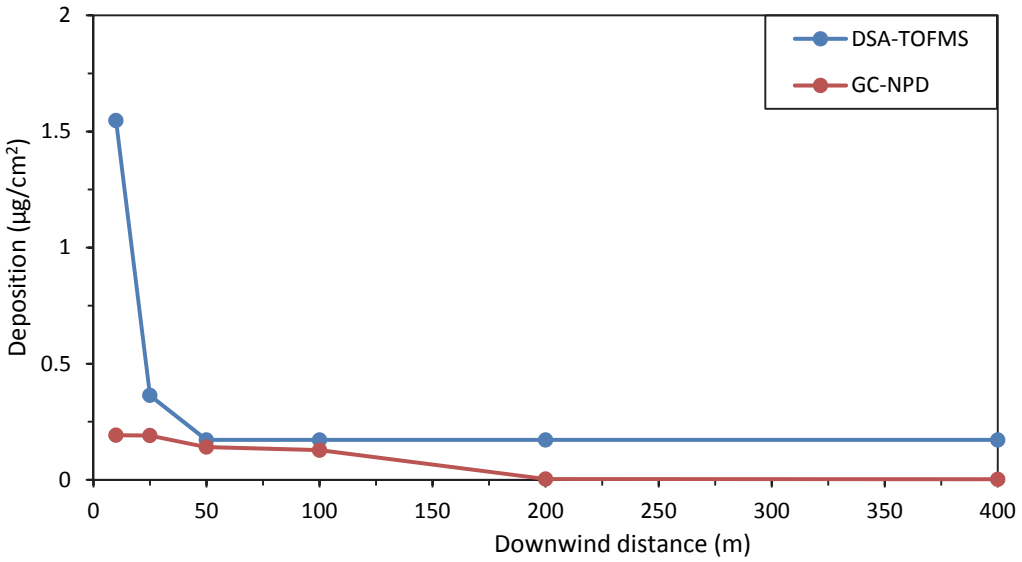


Figure 4.11: Experimentally measured deposition rates using two techniques: GC-NPD and DSA-TOFMS. The insert shows the GC-NPD concentration with a different scale. The results are only for samples taken from the central sampling line.

4.4.4 Model Sensitivity

Among the parameters that were investigated, the model was found to be most sensitive by far, to the droplet size distribution for both deposition (Figure 4.12) and airborne concentrations (Figure 4.13), with a very high variability with variation in droplet size especially closer to the application site. For lighter droplets the model predicted a higher concentration, suggesting that they are more likely to remain airborne, whilst larger droplets precipitate to the ground more readily. The results also showed that varying the evaporation rate (± 10%) did not have much of an impact on the predicted concentrations hence assuming that the spray mix had an evaporation rate similar to that of water should not have had much of an impact on the predicted results. Among the meteorological parameters tested (where the minimum and maximum values measured were compared to the averages used), the model was found to be more sensitive to changes in wind speed than the other parameters, with higher wind speed resulting in higher levels of spray drift, as expected. An ANOVA test revealed that indeed there was more variation in concentrations when changing the droplet size distribution ($p < 0.05$) than with any of the other parameters tested and that among the meteorological parameters, the model was more sensitive to wind speed.

These results agree with previous studies where droplet size was reported to be the most significant parameter affecting spray drift (Miller *et al*., 2011; Nuyttens *et al*., 2013) and that wind speed was reported to be the most significant meteorological parameter (Bird *et al*., 1996; Phillips and Miller, 1999). Hoffmann (2006) also performed some sensitivity studies with the AGDISP model and found that a 1.5 m/s increase in wind speed corresponded to a 100% increase in spray flux at 50 m. Another AGDISP model sensitivity study by Huang *et al*. (2010) concluded that wind speed is more significant than the droplet size. This could be because their simulation was for aerial application

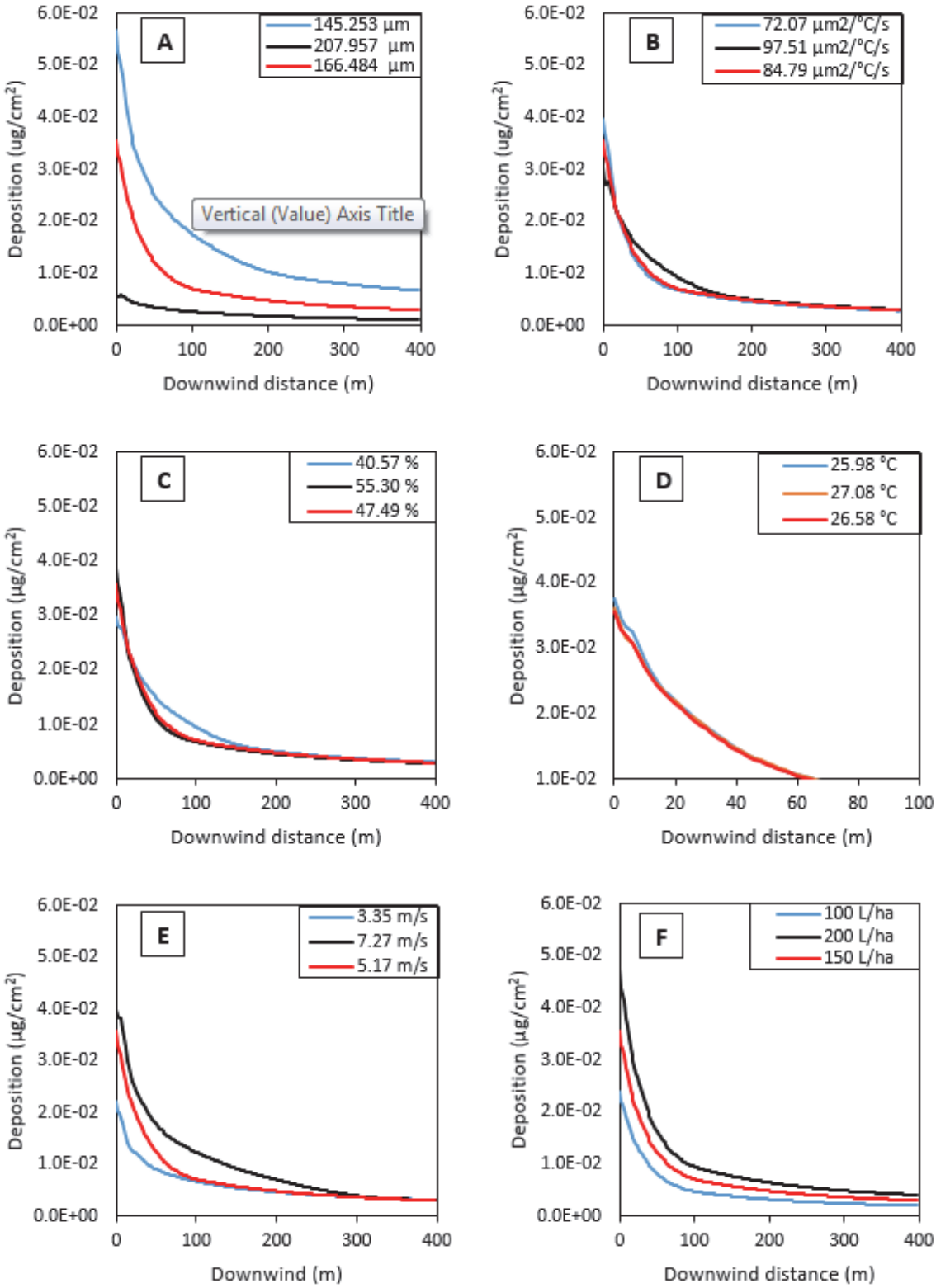


Figure 4.12: Sensitivity of the pesticide deposition rates predicted by the AGDISP model to six different input parameters (a) Droplet size (b) Evaporation rate (c) Relative humidity (d) Temperature (e) Wind speed (f) Application rate.

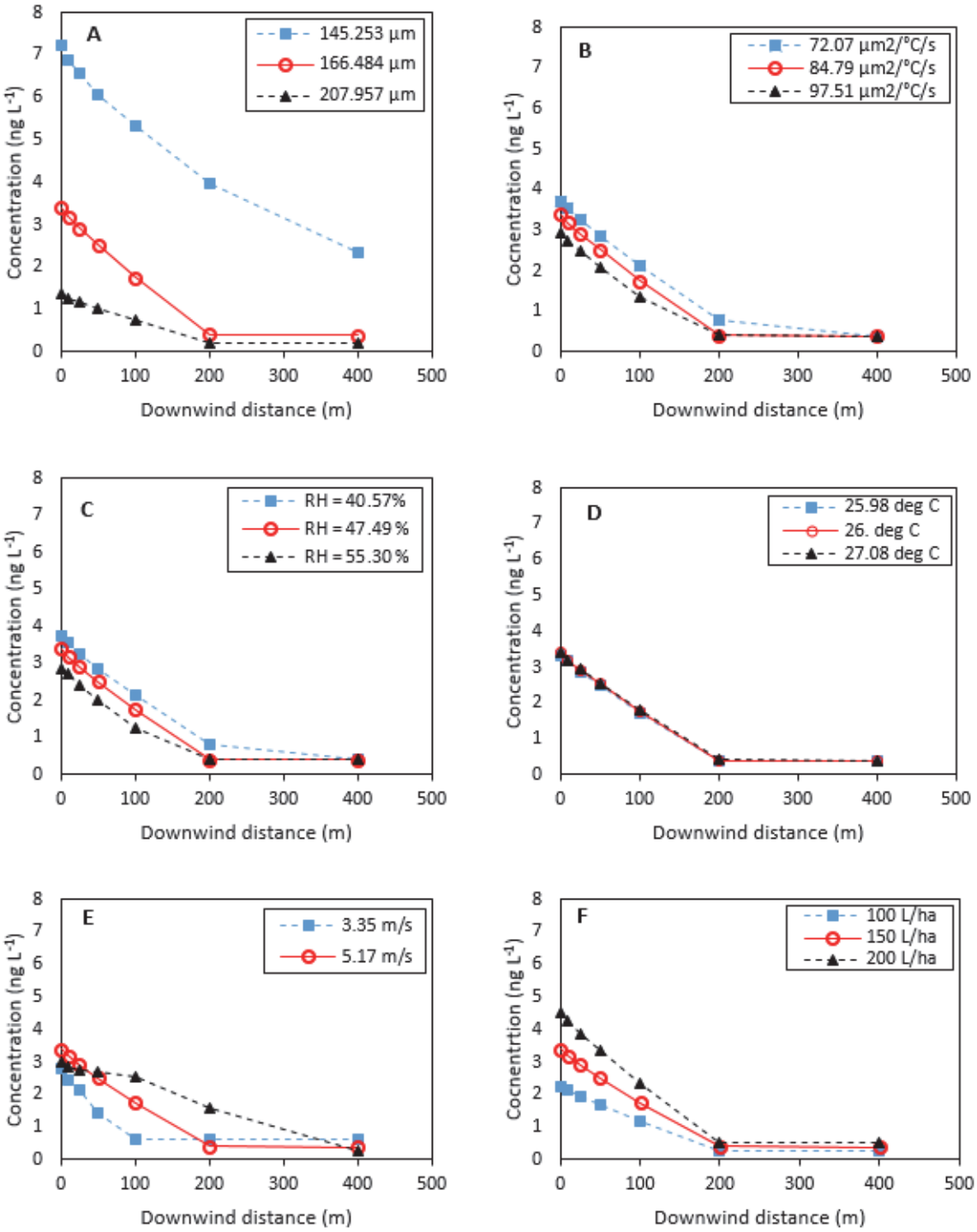


Figure 4.13: Sensitivity of the airborne concentrations of pesticides predicted at 1.5 m height by the AGDISP model to the variation in six different input parameters (a) Droplet size (b) Evaporation rate (c) Relative humidity (d) Temperature (e) Wind speed (f) Application rate.

while our study was for ground based application. In addition the range in variables tested differed: in our case we chose possible variations during our specific sampling campaign, whilst Huang *et al.* considered extremes. Consequently for droplet size their range was from very fine to very coarse, while ours ranged from 145 to 208 µm, and the maximum and minimum wind speeds were 0.45 to 6.71 m/s (Huang *et al.*) versus 3.35

to 7.27 m/s (this study). This shows that the most significant variable may be different for different pesticide application scenarios.

4.4.5 Scenario modelling results

With respect to the Letsitele scenario, the model could not perform the simulation because the spray volume rate or application rate was outside of the permissible limits of 0.0073 L/ha to 935.28 L/ha. In this scenario the application rate of 2000 L/ha was clearly far outside this range.

The predicted results for airborne concentrations of the different pesticides at a 1.5 m vertical height are shown in Figure 4.14 for the Bothaville scenario and in Figure 4.16 for the Komatipoort scenario. In the Bothaville scenario the concentrations were significantly higher than that obtained for the Komatipoort scenario and this can be attributed the different spray volumes used for the two areas. The higher spray volume rate in Bothaville implies that more of the pesticides are released during spraying, hence the higher airborne concentrations. These concentrations can provide a useful guide for assessing the risk that these pesticides can pose to nearby bystanders or animals either through inhalation or dermal exposure.

The simulated downwind deposition for the two areas is shown in Figure 4.15 for Bothaville and Figure 4.17 for Komatipoort. As expected, the downwind deposition is very low in the Komatipoort scenario compared to the Bothaville scenario, again being primarily due to the different spray volume rates. These results provide a useful guide for risk assessment studies to estimate possible contamination of nearby water resources, soil and other nearby agricultural crops.

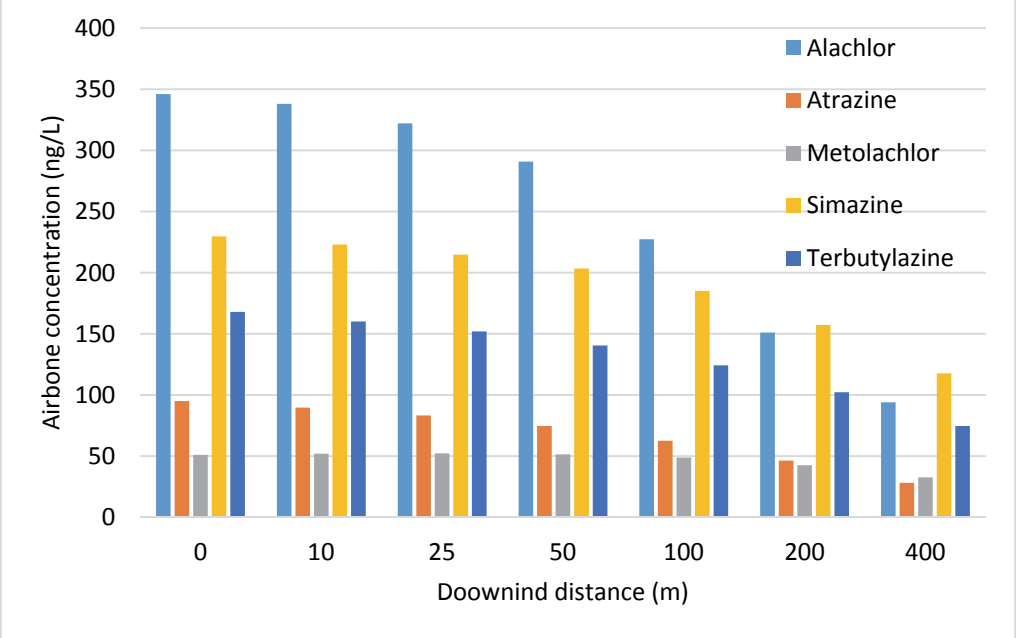


Figure 4.14: Worst case (fine droplet size) air concentrations predicted using the AGDISP model for different pesticides in Bothaville during pesticide application.

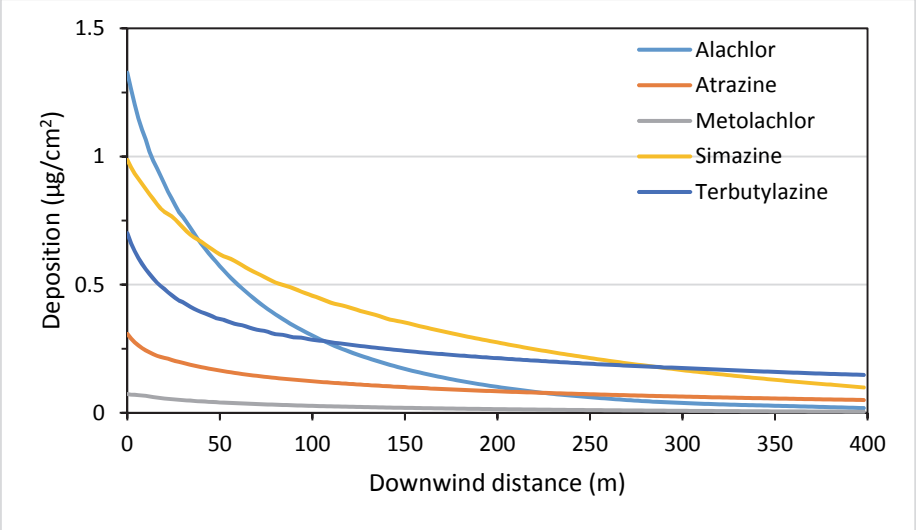


Figure 4.15: Worst case (fine droplet size) deposition of different pesticides predicted using the AGDISP model for different pesticides in Bothaville during pesticide application.

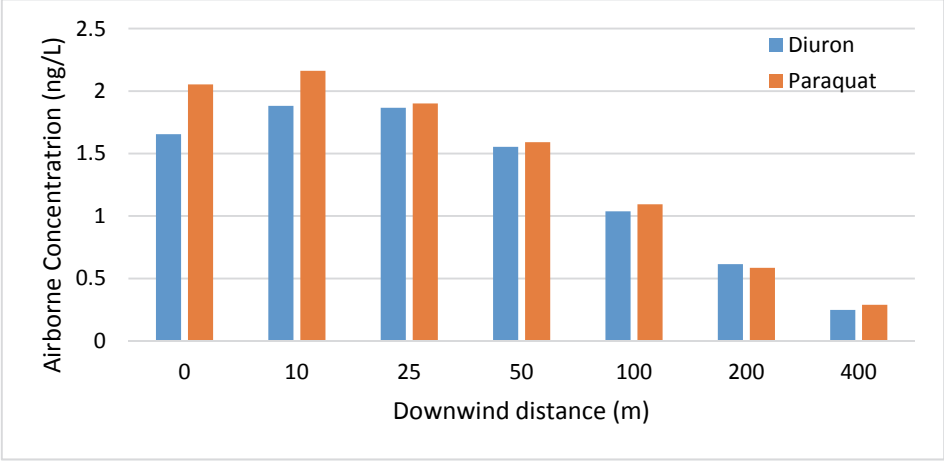


Figure 4.16: Worst case (fine droplet size) air concentrations predicted using the AGDISP model for different pesticides in Komatipoort during pesticide application.

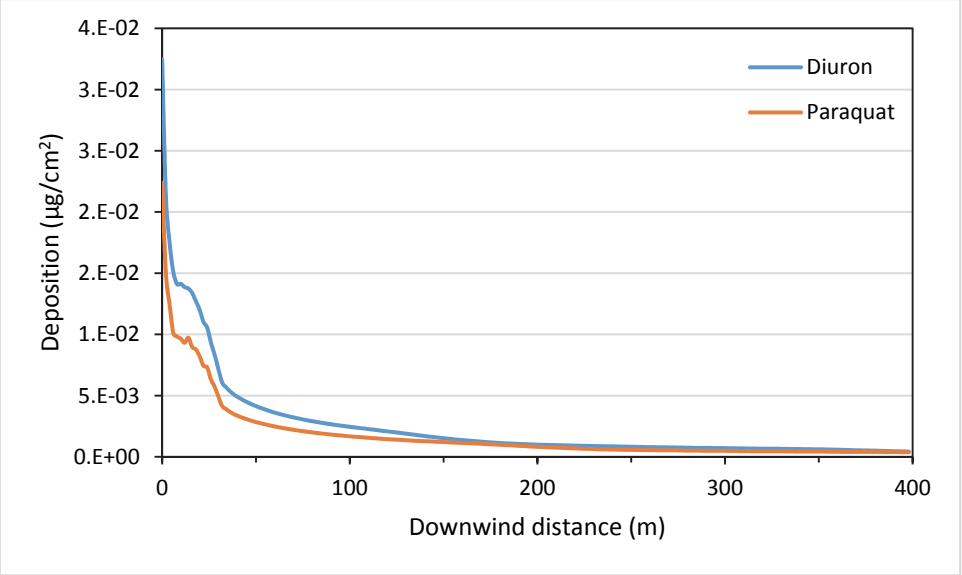


Figure 4.17: Worst case (fine droplet size) deposition of different pesticides predicted using the AGDISP model for different pesticides in Komatipoort during pesticide application.

4.4.6 Limitations of the AGDISP Model

Although the model showed good predictions in this study, previous studies that have done validations on the AGDISP model have reported some limitations on the ground application part of the model. Woodward *et al*. (2008) found that when compared to experimental data, the AGDISP 2003 ground application model over-predicted downwind deposition by a factor of 3.5-100. After doing some sensitivity studies and varying the humidity (which affects evaporation) they still found discrepancies between modelled and experimental values. They then recommended that there is a strong need to improve the evaporation algorithm in AGDISP. Similarly, Zabkiewicz *et al*. (2009) also found AGDISP to overestimate spray drift deposition.

Connell *et al*. (2012) found a number of discrepancies between the AGDISP (version 8.25) ground model and experimental data. They found a spray drift deposition ratio of model to experimental data to be between 1.5 and 12.4 from the different studies that they did. They also found that the measured flux was much higher than that calculated by AGDISP and they suggested that there is a need to improve the model so that it considers the wake effects from boom sprayers.

Although extensive modifications have been done on the model for aerial application, some validation studies for aerial applications have also reported some shortcomings of the model. Hoffmann (2006) found the AGDISP model to predict 1-6 times higher levels of spray materials compared to that measured by field samplers. Hoffmann *et al*. (2007) found that although the vertical deposition values for canopy heights between 0.3 and 0.8 m were comparable between field-collected and AGDISP (version 8.08) data, the model over-predicted by a factor of 1 the levels of spray drift at 1 m for the trials conducted at 0 or 1 m crop canopy heights. They also found the model to be not satisfactory for crop canopies that are >80% closed.

Schleier and Peterson (2010) showed that AGDISP and AgDRIFT were inadequate for estimating environmental concentrations from applications using ultra-low-volume sprayers. They concluded that the use of these models could result in an underestimation of exposures, but this could be partially because the researchers in this study used inputs from assumptions outlined in a different study to run the models.

It should also be noted that the model does not take localized turbulence (such as that due to nearby buildings) into account, which may impact on the results obtained. The results of any pesticide modelling study should be used with caution, and it is important to note that an over prediction is preferable to provide cautionary levels rather than an under prediction which would underestimate potential human health and environmental effects as a consequence of pesticide spray drift.

4.5 CONCLUSIONS

In this study, the active pesticide ingredient, namely atrazine, was successfully used to monitor downwind spray drift during pesticide application. This was due in part to the stability of atrazine in the atmosphere, with a half-life of approximately 1 day (De Rossi, 2010), as well as to its compatibility with the sampling and analytical method that was developed and employed.

For airborne spray drift monitoring, the PUF adsorbents proved to be suitable samplers for atrazine in the atmosphere. Although the extraction method used a fair amount of solvents, it gave very good recoveries. The Nitrogen Phosphorus Detector on the GC was also excellent for this application, being both selective and sensitive, which allowed for the determination of atrazine down to 0.138 pg/L at 400 m downwind. Satisfactory agreement was found between the modelled and experimental results when using the larger droplet size distribution, suggesting that coarse droplets were produced by the sprayer. We suggest, therefore, that the model can provide a good estimate of airborne spray drift concentrations which can be used for risk assessments or regulatory measures around sensitive areas. This, however, is provided one has reliable input parameters that are representative of the actual application conditions. Previous validation studies have shown variation in airborne spray drift of approximately two orders of magnitude between field and AGDISP modelled results (Teske *et al*., 2004; Woodward *et al*., 2008). In comparison, this study has shown promising correlations between experimental results and those predicted by the model. This improvement could be because the actual active pesticide ingredient was monitored instead of a tracer compound.

Deposition drift monitoring results did not show as good a correlation with the AGDISP model, with the model under-predicting pesticide deposition rates. It is recommended that further investigations be conducted in order to verify and fully elucidate this effect.

Although this study was focusing on pesticide spray drift of up to 400 m downwind, De Rossi (2010) stated that if •OH radical attack is the main degradation pathway, the pesticide can potentially travel up to 250-500 km in the atmosphere within a day under wind speeds of between 3-5 m/s. A further validation study which includes sampling at greater downwind distances should therefore be considered.

Sensitivity studies showed that the model is strongly dependent on the droplet size, where smaller droplets are more likely to remain airborne and drift further downwind whilst larger droplets easily deposit to the ground and are less likely to drift downwind (Fritz *et al*., 2010). The fact that droplet size was shown to be the major variable affecting off-target spray drift is supported by the results of previous studies (Hewitt *et al*., 2002a). Depending on the DSD input, the model may either overestimate or underestimate the atrazine concentration. It is therefore recommended that accurate determination of the actual DSD be considered in future studies using modern equipment such as laser diffraction spectrophotometry. In addition, the effect of the ingredients in the pesticide mix on the evaporation rate thereof requires further investigation.

In this study, the AGDISP pesticide air dispersion model was validated for the first time for use in South Africa. In contrast to other international validation studies conducted on this model, we used the active ingredient for model validation and validated the model at longer downwind distances.

4.6 RECOMMENDATIONS FOR FUTURE RESEARCH

- Analyse wool samples and compare results to those of this study
- Conduct additional scenario modelling studies for use in risk assessments in targeted study areas
- Extend spray drift sampling further than 400 m downwind (up to 20 km)
- Validate the model for local aerial applications
- Perform additional deposition rate validation experiments to verify the under prediction found in this study
- Use modern, accurate techniques for droplet size distributions and compare with that obtained using the MgO slides
- Determine the effect of pesticide mix ingredients on the evaporation rate of the solution
- The importance of spray nozzle maintenance and selection needs to be communicated to farmers
- General project recommendation: speciation analyses of metals found in water samples should be undertaken in order to assist with the elucidation of sources thereof.

4.7 ACKNOWLEDGEMENTS

Etienne van der Walt, Pietie van Niekerk and Annette Venter from the Agricultural Research Commission are sincerely thanked for allowing us to use their sampling equipment, for their active involvement and assistance during sampling, and for offering to assist with sample analysis in their laboratories. Perkin Elmer is thanked for providing access to the AxION DSA-TOFMS instrumentation. We are grateful to Justinus

Shadung and Ernst Bekker who also assisted with sampling. Finally we thank the Blignaut family in Standerton, particularly Sias, Peet, Hennie and Annette for allowing us to use their farm to carry out our sampling campaign.

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CHAPTER 5: ANDROGENIC ACTIVITY OF WATER AND SEDIMENT SAMPLES

by

R. Pieters and L. Powrie

Unit of Environmental Sciences and Management, North-West University
Potchefstroom, 2520

5.1 INTRODUCTION

5.1.1 Endocrine disrupting compounds

A variety of synthetic and natural occurring chemicals have been identified as endocrine disrupting compounds (EDCs). Such chemicals have the ability to interfere with endogenous hormone homeostasis (Rijk *et al*., 2012) which may, in turn, result in growth, development and reproductive complications in humans and wildlife. Mechanisms of action include the binding to and subsequent (in)activation of cellular hormone receptors, modifying hormone receptor levels as well as altering normal hormone levels by affecting biosynthesis, transport and metabolism (Aït-Aïssa *et al*., 2010; Rijk *et al*., 2012; McKinley, 2008). Of particular concern are chemicals that bind to oestrogen or androgen receptors in part because these receptors are highly conserved across species (Blake *et al*., 2010).

Concern have been expressed regarding the widespread use of agricultural pesticides during animal disease control and crop spraying since studies have indicated that several currently used pesticides have shown to be EDCs (Burger and Nel, 2008; McKinlay *et al*., 2008; Preda *et al*., 2012). In the case of pesticides with (anti)androgenic activity the normal transcription of androgen-dependant genes are altered which may result in genital malformations, a decrease in semen quality as well as cancers of reproductive organs in humans and animals which have been exposed to these chemicals (Ermler *et al*., 2010).

5.1.2 MDA-kb2 reporter gene bioassay

The androgen screening bioassay that was used in this study is the MDA-kb2 reporter gene bioassay developed by the US EPA (Wilson *et al.*, 2002). A breast cancer cell line, MDA-MB-453, was stably transformed with the MMTV.luciferase.neo reporter gene construct since it has shown to express high levels of functional, endogenous androgen receptors (AR). The principle of the bioassay is based on the inhibition or activation of nuclear AR.

Compounds that can act through the AR (AR-agonists), activate the MMTV luciferase reporter gene, inducing luciferase expression and when the enzyme's substrate, luciferin, is presented, bio-luminescence is emitted by the cells. The quantity of light

emitted by the cells is directly proportional to the concentration of AR-agonists that the cells were exposed to. The response from cells that received samples is expressed as equivalents of the response induced by the positive control, which is testosterone (T), i.e. T-equivalents (T-eq). AR-antagonists inhibit androgen induced luciferase expression. The response of the cells to the samples was compared to the solvent control (SC) in order to determine if AR-antagonists were present in the sample mixtures.

5.2 EXPERIMENTAL PROCEDURES

5.2.1 Sample collection and storage

5.2.1.1 Water samples

Water samples were collected in 1 L glass Schott bottles pre-washed with ethanol. The pH of the samples was adjusted to approximately 3. The samples were kept at 4˚C until the extraction process could commence. All extracted samples were kept at -20˚C.

5.2.1.2 Sediment samples

Composite sediment samples were collected and transported in high density poly-ethylene bottles (Nalgene) pre-washed with acetone and hexane. Three 250 mL bottles were collected at each site. Samples were stored at -20°C until the extraction protocol started.

Frozen samples were thawed and air dried at room temperature, protected from ultraviolet radiation break down. Dried sediment was grinded and sieved (0.5 mm mesh size) to obtain homogenous samples.

5.2.2 Chemical extraction

5.2.2.1 Water samples

For extraction and enrichment of potential oestrogen-like compounds a solid phase extraction (SPE) was performed according to the protocol described in the WRC's manual on bioassays for the detection of oestrogenic activity in water (De Jager *et al.*, 2012).

5.2.2.2 Sediment samples

Approximately 40 g of each site's sediment samples were extracted using pressurised liquid extraction (PLE), a method approved by the US EPA (Method 3545) (US EPA, 1996). The solvent was a 3:1 mixture of dichloromethane (DCM) and acetone and the temperature was set to 40°C to accommodate all possible

organic pollutants. A Dionex100 was used. Settings for the instrument were as follows:

Static time:	5 min
Flush volume:	60%
Purge time:	100 s
Static cycles:	2

The samples were evaporated to dryness and reconstituted to 1 mL with DCM. In order to remove insoluble particles the samples were subjected to solid phase extraction (SPE) (Method 3535A) (US EPA, 2007) (Sep-Pak Florisil, 3 cc: WAT020815). The cartridges were conditioned with 2 mL methanol before the samples were loaded. Each sample was eluted with 2 mL DCM. Methanol as well as DCM was collected into the same test tube. The samples were evaporated to dryness and reconstituted to 2 mL with DCM and transferred into recovery vials for the final clean-up process. To remove the sulphur from the extract, size exclusion with a gel permeation column (GPC) was performed. A standard mixture containing corn oil, phthalate, perylene, methoxychlor and sulphur were used to calibrate the GPC as well as to determine the retention time of each compound (Method 3640A) (US EPA, 1994) so that the fraction without the sulphur could be collected. Samples were evaporated to dryness and reconstituted to 2 mL with ethanol. The first 1 mL was provided to Dr N.H. Aneck-Hahn and Mrs M.C. Van Zijl (University of Pretoria) for the oestrogen bioassays. The remaining 1 mL was evaporated to dryness again and reconstituted to 1 mL with methanol for the androgen bioassay at the North-West University (Potchefstroom Campus).

5.3 MDA-KB2 REPORTER GENE BIOASSAY

5.3.1 Maintenance of cell culture

The cells were cultured in Leibovitz's L-15 medium (Gibco) supplemented with 10% foetal bovine serum (FBS) at 37°C in a humidified atmosphere with no CO_2 supplementation (Wilson *et al*., 2002).

5.3.2 Experimental procedure

Serial dilutions were made of the sample extracts as well as of testosterone (T) (positive control). The ratio for the sample dilutions was 1:1 and there were five dilutions for each sample. The samples were evaluated for their ability to activate AR or inhibit androgen binding. The concentrations of the positive control that was used in the test for activation varied between two bioassay periods (BAPs) as well as the T concentration that was included in the media during the test for inhibition. These differences were due to changes in the sensitivity of the cell line over the extended period of this project and are summarised in Table 5.1. The reason for this change is

still unknown since the laboratory environment and procedure stayed consistent throughout the study. According to the results from all three study areas it appears as if more activation responses were detected during BAP2. The BAP2 provided the benefit of more sensitive cells which could detect very small concentrations of testosterone-like compounds in the environmental samples. The limits of detection (Table 5.2) also indicates that the cells' responses during BAP2 were more sensitive by a factor of 10 000, for both the water (1.5×10^{-14} µg T/L) and sediment (8.0×10^{-7} µg T/g) matrices, compared to BAP1, which further supports the reliability of the bioassay results obtained during this part of the project.

Table 5.1: Testosterone concentrations used during two bioassay periods.

	Catchment	Sampling period	Matrix (W = Water; S = Sediment)	Activation T dilution series (µg/mL)	Inhibition T background (µg/mL)
Bioassay period 1	**Letsitele**	July 2011	W & S	1. 23.1 2. 5.8 3. 1.4 4. 3.6×10^{-1} 5. 9.0×10^{-2} 6. 2.3×10^{-2}	8.6×10^{-5}
		November 2011	W & S		
		August 2012	W		
	Vals & Renoster	July 2012	W & S		
		October 2012	W		
	Mzinti, Lomati & Ngweti	June 2012	W & S		
		September 2012	W		
Bioassay period 2	**Letsitele**	August 2012	S	1. 2.3×10^{-3} 2. 4.6×10^{-4} 3. 9.2×10^{-5} 4. 1.8×10^{-5} 5. 3.7×10^{-6} 6. 7.4×10^{-7}	3.91×10^{-5}
		November 2012	W & S		
		February 2013	W & S		
	Vals & Renoster	October 2012	S		
		January 2013	W & S		
		April 2013	W & S		
	Mzinti, Lomati & Ngweti	September 2012	S		
		December 2012	W & S		
		March 2013	W & S		

DHT: Dihydrotestosterone

Table 5.2: Limits of detection values for bioassay period 1 and bioassay period 2.

	Limits of detection	
	Water (µg T/L)	**Sediment (µg T/g)**
Bioassay period 1	1.8×10^{-10}	9.0×10^{-3}
Bioassay period 2	1.5×10^{-14}	8.0×10^{-7}

T: Testosterone

To determine activation, 30 000 cells were seeded into the inner 60 wells of a 96-well micro titre plate in medium that was supplemented with 10% dextran-charcoal treated FBS (Hyclone). Samples and standards were dosed in triplicate 48 h after initial seeding, and incubated another 48 h before luminescence was determined with a plate reader (Berthold multimode micro plate reader, model-LB941). The cells were first lysed with a lyse buffer and frozen at -80°C for approximately 10 minutes. When thawed, a solution that contained luciferin, was injected into each well by the plate reader. The solution consisted of 20 mM tricine,1.07 mM $Mg(CO_3)_4Mg(OH)_2$, 2.67 mM $MgSO_4 \bullet 7H_2O$, 0.1 mM EDTA, 33.3 mM DTT, 270 µM CoA, 470 µM Luciferin (Promega), 530 µM ATP (Wilson *et al*., 2002). Luminescence was captured as relative light units (RLU) which was used to express the samples' responses. RLU's were converted to relative response units, expressed as a percentage of the maximum response observed for T (% Tmax).

Bioassay-derived T-equivalents (T-eqs) were derived by using the regression equation for the appropriate T standard curve to calculate a concentration of T-eqs that produced a bioassay response equal in magnitude to that induced by the sample in question. The conversion to µg T-eqs was back-calculated based on the volume of extract assayed (i.e. 2.5 µL) and the degree of concentration during the extraction procedure (i.e. 20 g sediment concentrated into 1 mL of extract) (Koh *et al*., 2006).

To measure inhibition, the same protocol was followed as described above, with two changes: the solvent control (SC) was used as the reference control and the cells were seeded with a known T concentration in the media (Table 1) which resulted in cells with slightly activated ARs. The SC wells were seeded and dosed in triplicate with solvent (methanol) alone and no inhibition of androgen binding took place within these wells. The ability of the samples to inhibit existing cellular response was compared to that of the SC. In the case of inhibition the cellular response (RLUs) of the sample containing wells was expressed as a fraction of the response off the SC wells and is called fold inhibition (FI). Although Student t-test is a parametric test, it was employed on the non-parametric data to gauge if the observed inhibition of the sample extract is statistically significantly different to the response from the SC wells and whether the observed inhibition was due to natural variation or to exposure to inhibitive compounds.

5.4 VIABILITY ASSAY (MTT)

Sample cytotoxicity were assessed by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay as described by Mosmann (1983). The assay was conducted to prevent false negative responses since low luminescent responses cannot necessarily be contributed to the absence of AR agonists. It could be to toxicity of the extract destroying the cells. The assay is based on the principle that yellow MTT salts are metabolised to a blue formazan product by living cells. Formazan production was quantified spectrophotometrically to determine cell viability and the optical density of the solutions in each well was determined at 490 nm. If the percentage viability was below 80% the sample was

considered to be cytotoxic, which could have affected the reporter gene bioassay results.

5.5 RESULTS AND DISCUSSION

Results from each of the three study areas (Letsitele; Mzinti, Lomati and Ngweti; Vals- and Renoster River catchments) are presented separately. The surface water and sediment samples, collected from 2011 to 2013, were screened for (anti)androgen activity. The results are provided in Tables 5.3-5.8. The tables were created to contain one matrix, i.e. either water or sediment, but all of the sampling periods. Even though some tables contain apparently no data, i.e. no T-eq or FI, they were included because “no activity” is also a meaningful result. The results for the groundwater samples, which were collected in September, October and November of 2013, were included in the water result tables.

5.5.1 Letsitele Catchment

It was the only one of the three catchments that had data for 2011 also but for the sake of completeness, the 2011 results were included. Surface water and sediment samples were collected in July and November of 2011 as well as in August, November and February of 2012.

The water extracts’ results are presented in Table 5.3. Not one of the water samples from the Letsitele River catchment assayed showed any cytotoxicity. Compared to the SC the July 2011 sample LT1 caused significant inhibition of androgen binding as well as four samples of the November 2011 sampling trip. Sample LT1 (November 2011) significantly inhibited the AR, 1.45 times greater than the same sites’ sample from the July 2011 trip. The water samples collected in August of 2012 elicited androgenic responses (activation) in three instances. Extracts from samples LT3, LT4 and LT9 caused complete dose-response curves, i.e. their maximum response was comparable to the maximum response of the positive control, T (BAP1; Table 5.1). None of the groundwater samples from the September 2013 sampling event elicited any response from the MDA-kb2 cells.

The sediment samples collected during the same period elicited little androgenic responses from the cells (Table 5.4). The July 2011 sampling period showed one instance of activation. Extracts from site LT2 caused a response comparable to 20% of the maximum response of the positive control, T (BAP1; Table 5.1). The sample from site LT2 also caused significant inhibition of AR during the same sampling period (July 2011). This potentially implies that both androgenic and anti-androgenic compounds were present in the sample and that competition resulted in both activation of the AR as well as inhibition of androgen binding. There were nine cases of inhibition in total. Sample LT8 (November 2011), for example, caused significant inhibition of androgen binding and elicited a response from the cells almost two times (FI = 1.93) lower compared to that of the SC.

Table 5.3: Bioassay results for androgen activation (ng/L T-eq) and fold inhibition in surface and groundwater samples from the Letsitele River Catchment.

Site	July 2011		November 2011		August 2012		November 2012		February 2013		September 2013	
	Act	Inhib	Act	Inhib	Act	Inhib	Act	Inhib	Act	Inhib	Act	Inhib
LT1	-	1.01	-	1.47	-	-	-	-	-	-	N/S	
LT2	-	-	-	1.36	-	-	-	-	-	-	N/S	
LT3	-	-	-	-	$8.5x10^{-3} \pm 2.4x10^{-3}$	-	-	-	-	-	N/S	
LT4	-	-	-	-	$7.6x10^{-3} \pm 5.0x10^{-4}$	-	-	-	-	-	N/S	
LT5	-	-	-	-	-	-	-	-	-	-	N/S	
LT6	-	-	-	-	-	-	-	-	-	-	N/S	
LT7	-	-	-	1.39	-	-	-	-	-	-	N/S	
LT8	-	-	-	1.30	-	-	-	-	-	-	N/S	
LT9	-	-	-	-	$5.2x10^{-3} \pm 1.4x10^{-3}$	-	-	-	-	-	N/S	
LT10	-	-	-	-	-	-	-	-	-	-	N/S	
BM	N/S		N/S		N/S		N/S		N/S		-	-
FM	N/S		N/S		N/S		N/S		N/S		-	-
SL	N/S		N/S		N/S		N/S		N/S		-	-

Table 5.4: Bioassay results for androgen activation (ng/L T-eq) and fold inhibition in sediment samples from the Letsitele River Catchment.

Site	July 2011		November 2011		August 2012		November 2012		February 2013	
	Act	Inhib	Act	Inhib	Act	Inhib	Act	Inhib	Act	Inhib
LT1	-	-	-	-	-	-	-	-	-	-
LT2	2.1 ± 0.2	1.28	-	-	-	-	-	-	-	-
LT3	-	1.27	-	1.72	-	-	-	-	-	-
LT7	-	1.17	-	1.46	-	-	-	-	-	-
LT8	-	1.68	-	1.93	-	-	-	-	-	-
LT9	-	-	-	1.57	-	-	-	-	-	-
LT10	-	1.34	-	-	-	-	-	-	-	-

Samples LT3, LT7 and LT8 from the November 2011 sampling trip respectively caused an inhibition response from the cells 1.35, 1.25 and 1.15 times greater compared to the same July 2011 samples. Both the August 2012 and November 2012 samples from site LT3 were cytotoxic with cell viability of 74% and 59% respectively.

There are no discernible patterns of (anti)androgenic activity between matrices of the same sampling period or between samples from different sampling events. Most of the activity occurred during July 2011 and November 2011. The few cases of activation caused by sediment and water samples occurred during the winter season (July 2011 and August 2012). Low flow and subsequent higher aquatic concentrations of AR-agonists potentially resulted in positive responses elicited by the MDA-kb2 cells.

The chemical screening analysis of the samples collected in the Letsitele River Catchment rendered five pesticides which were detected most frequently (e.g. diphenylamine, imazalil, imidacloprid, propiconazole and thiabendazole) (CHAPTER 3:). Thiabendazole, imidacloprid and diphenylamine had the highest number of detections in both the water and sediment samples. Studies have proved all of these pesticides, except for diphenylamine, to have endocrine disrupting properties (CHAPTER 9:). Imazalil and propiconazole have shown to be inhibitors of androgen receptor binding but these pesticides were rarely detected concurrently with anti-androgenic responses. It is an uncertainty whether pesticides were responsible for the elicited responses or whether other compounds (e.g. industrial and agricultural waste) could have caused the activity.

5.5.2 Vals and Renoster Catchments

Results for both matrices, sampled during July and October of 2012 as well as those sampled in January, April and October of 2013, are included in Table 5.5 and Table 5.6. There was no evidence of (anti)androgenic activity in either the surface water or the groundwater samples. All of the July 2012 samples except for RN1 and RN4 were cytotoxic (viability < 80%). Sample VL3 were cytotoxic in July 2012 as well as January 2013 with cell viability of 60% and 42% respectively. The sediment samples from the same site also caused cytotoxicity during the July 2012 and April 2013 sampling events.

All of the sediment sampled in July 2012 were cytotoxic and also elicited androgenic responses from the cells, indicating the presence of pollutants that bind to the testosterone receptor in the cells and cause subsequent expression of the luciferase gene. Extracts from samples VL3 and RN1 elicited complete dose-response curves, i.e. their maximum response was comparable to the maximum response of the positive control, T. These two samples also caused the most severe cytotoxicity compared to the other samples with cell viability of 64% and 54% respectively. Samples VL1, RN3 and RN4 had maximum responses comparable to the 50% maximum response of the reference while the highest response elicited by VL2 approximated 20% of the positive control's response (BAP1; Table 5.1).

Samples VL2 (October 2012) as well as RN1, RN3 and RN4 (January 2013) activated nuclear AR and instigated responses comparable to 20% of the maximum response of the DHT-reference (BAP2; Table 5.1). The responses comparable to 20% of the maximum response of the reference for all of the Vals- and Renoster River sediment samples have been presented in table 6 for the sake of consistency. Only two instances of significant AR inhibition, compared to the SC, occurred from the October 2012 samples (VL1 and RN4). Sample VL2, collected during the July 2012, January 2013 and April 2013 sampling events, caused slight cytotoxicity.

A potential reason for the high androgenic activity in the sediment samples may be that the sediment from July 2012, October 2012 and January 2013 had pollutants trapped which caused androgenicity, whereas water from the same periods did not have the same effect on the cells.

The chemical screening analysis rendered alachlor, atrazine, imidacloprid, simazine and terbuthylazine as the five pesticides which occurred most frequently (CHAPTER 3:). Studies have indicated that all of these pesticides have endocrine disrupting properties. Atrazine has shown to be an inhibitor of androgen binding (CHAPTER 9:). Atrazine, terbuthylazine and simazine were by far detected most frequently in both matrices and can potentially be held responsible for the activity which occurred in the sediment samples.

Table 5.5: Bioassay results for androgen activation (Act: ng/L T-eq) and inhibition (Inhib) in surface and groundwater samples from the Vals (VL) and Renoster (RN) River Catchments.

Site	July 2012		October 2012		January 2013		April 2013		October 2013	
	Act	Inhib	Act	Inhib	Act	Inhib	Act	Inhib	Act	Inhib
VL1	-	-	-	-	-	-	-	-	N/S	
VL2	-	-	-	-	-	-	-	-	N/S	
VL3	-	-	-	-	-	-	-	-	N/S	
RN1	-	-	-	-	-	-	-	-	N/S	
RN3	-	-	-	-	-	-	-	-	N/S	
RN4	-	-	-	-	-	-	-	-	N/S	
VJK	N/S		N/S		N/S		N/S		-	-
KOP	N/S		N/S		N/S		N/S		-	-
KSD	N/S		N/S		N/S		N/S		-	-

Table 5.6: Bioassay results for androgen activation (Act: ng/L T-eq) and inhibition (Inhib) in sediment samples from the Vals (VL) and Renoster (RN) River Catchments.

Site	July 2012		October 2012		January 2013		April 2013	
	Act	Inhib	Act	Inhib	Act	Inhib	Act	Inhib
VL1	29.4 ± 7.4	-	-	1.15	-	-	-	-
VL2	44.7 ± 24.8	-	$4.8x10^{-3} \pm 5.6x10^{-3}$	-	-	-	-	-
VL3	79.2 ± 3.7	-	-	-	-	-	-	-
RN1	50.9 ± 16.3	-	-	-	$3.7x10^{-4} \pm 1.5x10^{-4}$	-	-	-
RN3	39.0 ± 25.3	-	-	-	$1.5x10^{-4} \pm 1.2x10^{-4}$	-	-	-
RN4	55.2 ± 23.7	-	-	1.46	$1.7x10^{-4} \pm 2.8x10^{-5}$	-	-	-

5.5.3 Lomati Catchment

Surface water and sediment samples were collected during the months of June, September and December of 2012 as well as during March of 2013. Groundwater samples were collected during November 2013. The assay results for the water and sediment samples are presented in Table 5.7 and Table 5.8, respectively.

Surface water samples collected from sites NK1 and NK6 in December 2012 and from sites NK5 and NK6 in March 2013 elicited responses comparable to 20% of the maximum response of the reference (BAP2; Table 5.1). The responses were very low compared to the activation responses elicited by samples from the other study areas. The T-eq values in Table 5.7 and Table 5.8 were therefore presented in ng/L. These low responses further validate the high sensitivity of the MDA-kb2 reporter gene bioassay. None of the samples inhibited androgen binding. Samples NK3 and NK3Tap from the June 2012 sampling event showed to be cytotoxic with cell viability of 64% and 54% respectively.

The sediment samples were responsible for several activation responses which were all comparable to 20% of the maximum response of the T (BAP2; Table 5.1). Only sample NK6 from the September 2012 sampling event activated AR. All of the samples from the March 2013 sampling event, except for NK2 and NK3, elicited androgenic responses. Both surface water and sediment samples NK5 and NK6 elicited androgenic activity in March 2013. Samples NK5 and NK2 significantly inhibited androgen binding in September 2012 and March 2013. Sediment sample NK3 collected during June 2012 was cytotoxic (just like the water sample from the same sampling event) as well as the sample collected during March 2013 from the same site.

The chemical screening analysis rendered atrazine, carbofuran, imidacloprid, terbuthylazine and thiabendazole as the pesticides which were detected most frequently (CHAPTER 3:). All of these pesticides have been proven to have endocrine disrupting properties to some degree (CHAPTER 9:). Atrazine has been classified as a category 1 EDC and has been reported to be an inhibitor of androgen binding. Only two instances of anti-androgenicity occurred from samples collected in the Mzinti and Lomati River catchments which may potentially be ascribed to entrapment of endocrine disrupting pollutants in the sediment since the water samples did not elicit the same response. None of the detected and quantified pesticides have been proven to have androgenic activity. Further investigation regarding the NK3 site's cytotoxicity may be worthwhile.

Table 5.7: Bioassay results for androgen activation (Act: ng/L T-eq) and (Inhib) in surface and groundwater samples from the Lomati River catchment.

Site	June 2012		September 2012		December 2012		March 2013		November 2013	
	Act	Inhib	Act	Inhib	Act	Inhib	Act	Inhib	Act	Inhib
NK1	-	-	-	-	$2.8x10^{-8} \pm 7.0x10^{-9}$	-	-	-	N/S	
NK2	-	-	-	-	-	-	-	-	N/S	
NK3	-	-	-	-	-	-	-	-	N/S	
NK3Tap	-	-	-	-	-	-	-	-	N/S	
NK4	-	-	-	-	-	-	-	-	N/S	
NK5	-	-	-	-	-	-	$2.0x10^{-8} \pm 1.5x10^{-8}$	-	N/S	
NK6	-	-	-	-	$2.2x10^{-8} \pm 8.8x10^{-9}$	-	$8.1x10^{-9} \pm 3.0x10^{-9}$	-	N/S	
GW	N/S		N/S		N/S		N/S		-	-
KC	N/S		N/S		N/S		N/S		-	-
RH	N/S		N/S		N/S		N/S		-	-

Table 5.8: Bioassay results for androgen activation (Act: ng/L T-eq) and (Inhib) in sediment samples from the Lomati River catchment.

Site	June 2012		September 2012		December 2012		March 2013	
	Act	Inhib	Act	Inhib	Act	Inhib	Act	Inhib
NK1	-	-	-	-	-	-	$6.6x10^{-19} \pm 7.4x10^{-19}$	-
NK2	-	-	-	-	-	-	-	1.13
NK3	-	-	-	-	-	-	-	-
NK4	-	-	-	-	-	-	$1.2x10^{-6} \pm 2.0x10^{-6}$	-
NK5	-	-	-	1.26	-	-	$3.8x10^{-6} \pm 6.6x10^{-6}$	-
NK6	-	-	$8.8x10^{-2} \pm 2.3x10^{-2}$	-	-	-	$9.7x10^{-17} \pm 1.6x10^{-16}$	-

5.6 CONCLUSIONS AND RECOMMENDATIONS

Results indicate some androgen activity in some of the samples from the Letsitele River catchment, but there is no consistency in the behaviour elicited from the cells. Furthermore it seems likely that AR antagonists were present in water and sediment samples especially during the months of July and November of 2011. The Free State sediment samples from the Vals and Renoster rivers clearly elicited androgenicity. The water samples, however, seemed to contain either no quantifiable pollutants that would bind to the androgen receptor. The Mzinti, Lomati and Ngweti River Catchment samples caused very little anti-androgen activity in the cells but the assay resulted in a few cases of androgenicity.

Currently no standards or guidelines for testosterone in water or sediment exist. There is therefore no regulatory way of knowing at what levels testosterone may have chronic health effects in humans and animals. None of the (anti)androgenic responses elicited throughout this study can therefore be discussed in terms of potential health effects. This situation emphasizes the great need for further EDC research, specifically for androgens and androgen-like compounds. In an attempt to explain the elicited responses, the MDA-kb2 bioassay data must be compared to detected pesticides in the catchment which have been declared as EDCs or which are considered as possible EDCs. Other land uses and/or industries in the various catchments must also be considered as possible culprits for the positive responses.

The MDA-kb2 reporter gene bioassay may only be used as a screening tool in order to determine the presence or absence of pollutants with (anti)androgenic properties. Since the assay can therefore not be used to identify such pollutants it is furthermore important to integrate the bioassay results with the chemical analysis results. Some of the detected and quantified pesticides potentially caused the elicited responses but comparison of the data has not rendered any discernible patterns between matrices of the same sampling period or between samples from different sampling events. It is possible that other contaminants with ED properties were also present in the samples which caused the responses.

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CHAPTER 6: OESTROGENIC ACTIVITY OF WATER AND SEDIMENT SAMPLES

by

N.H. Aneck-Hahn, M.C. Van Zijl and C. de Jager

Environmental Chemical Pollution and Health Research Unit, Faculty of Health Sciences, University of Pretoria

6.1 INTRODUCTION AND OBJECTIVES

In-vitro bioassays have been widely used to determine if chemicals have endocrine disrupting activity. Many of these assays however have limited usefulness and are not freely available as a scientific tool (Wilson *et al.*, 2004). A suite of bioassays is therefore recommended when testing for endocrine disrupting activity (Beresford *et al.*, 2000).

6.1.1 The recombinant yeast *oestrogen* screen (YES)

A recombinant yeast strain was developed in the Genetics Department at Glaxo for use in a test to identify compounds that can interact with the human oestrogen receptor alpha (ERα) (Routledge and Sumpter, 1996). Yeast cells were transfected with the ERα gene, together with expression plasmids, containing oestrogen-responsive elements and the lac-Z reporter gene encoding the enzyme β-galactosidase. The yeast cells are incubated in a medium containing the test substance, vehicle control and 17β-estradiol (E_2) positive control respectively, together with the chromogenic substrate, chlorophenol red-β-d-galactopyranoside (CPRG). Active ligands, which bind to the receptor, induce β-galactosidase (β-gal) expression that causes the CPRG (yellow) to change into a red product that can be measured by absorbance (Routledge and Sumpter, 1996).

6.1.2 The T47D-KBluc reporter gene assay

The United States Environmental Protection Agency (US EPA) developed an oestrogen-dependent stable cell line. The T47D human breast cancer cells, which contain both endogenous ERα and ERβ, were transfected with an oestrogen-responsive element (ERE) luciferase reporter gene construct. This provides an *in vitro* system that can be used to evaluate the ability of chemicals to modulate the activity of oestrogen-dependent gene transcription. In principle, compounds enter the cell; oestrogen receptor ligands bind to the ER; two ligand-bound receptors dimerize and bind coactivators; then the dimmer binds to the ERE on the reporter gene construct and activates the luciferase reporter gene. The presence of the luciferase enzyme can then be assayed by measuring the light produced when the enzyme substrate, luciferin, and appropriate cofactors are added. The amount of light produced is relative to the degree of oestrogenic activity of the test chemical. When

testing chemicals using the T47D-KBluc cells, an oestrogen is defined as a chemical that induced dose dependent luciferase activity, which could be specifically inhibited by the anti-oestrogen ICI. Agonists stimulate luciferase expression and are compared to the vehicle control (media plus ethanol) or to the relative response of their respective E_2 control. Anti-oestrogens block the E_2-induced luciferase expression, which is compared to the E_2 control (Wilson *et al.*, 2004).

6.2 EXPERIMENTAL PROCEDURES

6.2.1 Sample collection and storage

6.2.1.1 Water samples

Water samples were collected in 1 L glass Schott bottles (Cat. No. 21 802 54 56, Merck) pre-washed with ethanol (Cat. No. 27,0741, Sigma-Aldrich). The pH of the samples was adjusted to approximately 3. The samples were kept at 4˚C until extracted. All extracted samples were kept at -20˚C.

6.2.1.2 Sediment samples

Composite sediment samples were collected and transported in high density poly-ethylene bottles (Nalgene). Three 250 mL bottles were collected at each site. Samples were stored at -20°C until the extraction protocol started. Frozen samples were thawed and air dried at room temperature, protected from ultraviolet radiation break down. Dried sediment was grinded and sieved (0.5 mm mesh size) to obtain homogenous samples.

6.2.2 Extraction procedure

6.2.2.1 Water samples

For extraction and enrichment of potential oestrogen-like compounds a solid phase extraction (SPE) was performed according to the protocol described in the WRC's manual on bioassays for the detection of oestrogenic activity in water (De Jager *et al*., 2011).

6.2.2.2 Sediment samples

Approximately 40 g of sediment sample was extracted using pressurised liquid extraction (PLE). This is a US EPA approved method (Method 3545) (US EPA, 1996). The solvent was a 3:1 mixture of dichloromethane (DCM) and acetone and the temperature was set to 40°C to accommodate all possible organic pollutants. A Dionex100 was used. Settings for the instrument were as follows:

Static time: 5 min

Flush volume: 60%

Purge time: 100 s

Static cycles: 2

The extract was concentrated to 1 mL and SPE (Sep-Pak Florisil, 3 cc: WAT020815) was used to remove insoluble particles. The cartridges were conditioned with 2 mL methanol, before the samples were loaded. Each sample was eluted with 2 mL DCM. Methanol as well as DCM was collected into the same test tube. The samples were evaporated to dryness and reconstituted to 2 mL with DCM and transferred into recovery vials for the final clean-up process. To remove the sulphur from the extract, size exclusion with a gel permeation column (GPC) was performed. A standard mixture containing corn oil, phthalate, perylene, methoxychlor and sulphur is used to calibrate the GPC as well as to determine the retention time of each compound (US EPA, 1994) so that the fraction without the sulphur could be collected. Samples were evaporated to dryness and reconstituted to 2 mL ethanol. 1 mL was provided to University of Pretoria for the oestrogen bioassays. The remaining 1 mL was evaporated to dryness again and reconstituted to 1 mL methanol for the androgen bioassay at the North-West University (Potchefstroom Campus).

6.2.3 YES assay

The YES assay was performed according to the assay procedure described in the WRC EDC toolbox manual (De Jager et al., 2011).

6.2.3.1 Maintenance of cell culture

The stock cultures and growth medium were prepared using medium components described in the WRC toolbox (De Jager et al., 2011). Short term stock cultures were used to prepare the assay medium for the experimental procedure. The growth medium was inoculated with 125 µL of the 10x concentrated yeast stock and incubated at 28°C in a rotating water bath at 150-155 upm until turbid.

6.2.3.2 Experimental procedure

Serial dilutions were made of the water and sediment sample extracts and controls, in 96 well microtiter plates (Cat. No. 95029780, Labsystems). 100 µL of the solvent, ethanol (Cat. No. 27,0741, Sigma-Aldrich) was placed in wells 2-12 on the plate. 200 µL of the sample extract was placed into the first well and this was serially diluted (100 µL) across the plate, using a multichannel pipette. 10 µL Aliquots were then transferred to a 96 well, optically flat bottom microplate (Cat. No. 95029780, Labsystems). This was allowed to evaporate to dryness on the assay plate. Aliquots (200 µL) of the assay medium containing the yeast and chromogenic substrate

(CPRG) were then dispensed into each sample well. Each plate contained at least one row of blanks (assay medium and solvent ethanol) and a standard curve for E_2 (Cat. No. E8875, Sigma) ranging from $1x10^{-8}$ M to $4.8x10^{-12}$ M (2.724 µg/L to 1.3 ng/L) which was extended to a concentration of $1.19x10^{-15}$ M ($3.24x10^{-13}$ g/L). The plates were sealed with parafilm (Cat. No. P7793, Sigma) and placed in a naturally ventilated incubator (Heraeus, B290) at 32°C for 3 to 5 days. After 3 days incubation the colour development of the medium was checked for 3 days (day 3 to 5) at an absorbance (abs) of 540 nm for colour change and 620 nm for turbidity of the yeast culture. The absorbance was measured on a Titertek Multiskan MCC/340 plate reader to obtain data with the best contrast. After incubation the control wells appeared light orange in colour, due to background expression of β-galactosidase and turbid due to the growth of the yeast. Positive wells were indicated by a deep red colour accompanied by yeast growth. Clear wells, containing no growth indicated lysis of the cells and colour varied from yellow to light orange. All experiments were performed in triplicate. The following equation was applied to correct for turbidity:

Corrected value = test abs (540nm) - [test abs (620nm) - median blank abs (620nm)]

The 17β-estradiol standard curve was fitted (sigmoidal function, variable slope) using Graphpad Prism (version 4), which calculated the minimum, maximum, slope, EC50 value and 95% confidence limits. The detection limit of the yeast assay was calculated as absorbance elicited by the solvent control (blank) plus three times the standard deviation. The estradiol equivalents (EEq) of the water and sediment samples were interpolated from the estradiol standard curve and corrected with the appropriate dilution factor for each sample.

6.2.4 The T47D-KBluc reporter gene assay

The water and sediment extracts were processed according to the protocol described in the WRC toolbox (De Jager et al., 2011).

6.2.4.1 Maintenance of cell cultures

T47D-KBluc cells were maintained in RPMI growth media (Cat. No. R8755, Sigma) supplemented with 2.5 g/L glucose, 10 mM HEPES, 1 mM sodium pyruvate, 1.5 g/L $NaHCO_3$, 10% fetal bovine serum (FBS) (characterized, Cat. No. SH30071.03, Hyclone, Separations, SA), 100 µg/mL penicillin, 100 U/mL streptomycin and 0.25 µg/mL amphotericin B (Cat. No. 15240-062, Gibco, Scientific Group, SA). One week prior to the assay, cells were placed in growth media modified by replacement of 10% FBS with 10% dextran-charcoal treated FBS (Cat. No. SH30068.03, Hyclone, Separations, SA) excluding antibiotic supplements.

6.2.4.2 Experimental procedure

Cells were seeded at 5 x 10^4 cells per well in 96-well luminometer plates and allowed to attach overnight. Dosing dilutions were prepared in growth media containing 5% dextran-charcoal treated FBS and vehicle (ethanol) did not exceed 0.2%. Each plate contained agonist positive control (E_2), negative control (vehicle only), antagonist control (E_2 plus ICI) and background control (vehicle plus ICI). Each sample was tested alone as well as in the presence of 0.1 nM E_2 or ICI. Cells were incubated 24h with 100 µL/well dosing solution at 37°C, 5% CO_2.

After the incubation period, cells were washed with phosphate buffered saline at room temperature and lysed with 25 µL lysis buffer (Cat. No. E3971, Promega, Whitehead Scientific, SA). Luciferase activity was determined using a LUMIstar OPTIMA luminometer and quantified as relative light units. Each well received 25 µL reaction buffer (25 mM glycylglycine, 15 mM $MgCl_2$, 5 mM ATP, 0.1 mg/mL BSA, pH 7.8), followed by 25 µL 1 mM D-luciferin 5s later. Relative light units were converted to a fold induction above the vehicle control value.

The E_2 standard curve was fitted (sigmoidal function, variable slope) using Graphpad Prism (version 4), which calculated the minimum, maximum, slope, EC_{50} value and 95% confidence limits. The EEq of extracts with greater than a twofold induction above the vehicle control were interpolated from the estradiol standard curve and corrected with the appropriate dilution factor for each sample.

6.3 RESULTS

6.3.1 Letsitele Catchment

The results obtained with the YES and T47D-KBluc assays for the Letsitele catchment are tabulated in Table 6.1 (water) and Table 6.2 (sediment). No anti-oestrogenic activity was detected in any of the samples and cytotoxicity was only detected in one of the sediment samples (LT2, Feb 2013) in the T47D-KBluc assay. Oestrogenic activity ranged between below the detection limit to 0.58 ng/L for water and below detection limit to 14.68 ng/kg for sediment samples.

6.3.2 Lomati Catchment

The results obtained with the YES and T47D-KBluc assays for the Lomati catchment are tabulated in Table 6.3 (water) and Table 6.4 (sediment). Oestrogenic activity ranged between below the detection limit to 0.42 ng/L for water and below detection limit to 65.04 ng/kg for sediment samples. No anti-oestrogenic activity was detected in any of the samples.

6.3.3 Vals and Renoster Catchments

The results obtained with the YES and T47D-KBluc assay for the Vals and Renoster catchments are tabulated in Table 6.5 (water) and Table 6.6 (sediment). Oestrogenic activity ranged between below the detection limit to 6.78 ng/L for water and below detection limit to 254.95 ng/kg for sediment samples. Anti-oestrogenic activity was detected in VL3 water samples in July and October 2012.

6.3.4 Additional water samples

The results obtained with the YES and T47D-KBluc assay for the additional water samples are tabulated in Table 6.7. Oestrogenic activity ranged between below the detection limit to 0.35 ng/L and no anti-oestrogenic activity was detected.

Table 6.1: Oestrogenic activity in Letsitele water samples using the YES and T47D-KBluc reporter gene assay

Site	Water: EEq (ng/L)									
	July 2011		November 2011		August 2012		November 2012		February 2013	
	YES	T47D-KBluc	YES	T47D-KBluc	YES	T47D-KBluc	YES	T47D-KBluc	YES	T47D-KBluc
LT1	<dl	0.12 ± 0.01	<dl	0.52 ± 0.14	<dl	0.04 ± 0.05	No sample	No sample	No sample	No sample
LT2	<dl	0.33 ± 0.08	<dl	0.19 ± 0.02	<dl	0.04 ± 0.05	<dl	0.05 ± 0.01	No sample	No sample
LT3	<dl	0.08 ± 0.02	No sample	No sample	<dl	0.002 ± 0.001	<dl	0.29 ± 0.07	<loq	0.07 ± 0.02
LT4	No sample	No sample	<dl	0.17 ± 0.02	<dl	0.01 ± 0.01	<dl	0.04 ± 0.01	<loq	0.24 ± 0.04
LT6	<dl	0.01 ± 0.003	<dl	0.02 ± 0.003	No sample	No sample	No sample	No sample	No sample	No sample
LT7	<dl	0.11 ± 0.01	<dl	0.08 ± 0.01	No sample	No sample	No sample	No sample	No sample	No sample
LT8	<dl	0.58 ± 0.09	<dl	0.11 ± 0.02	No sample	No sample	No sample	No sample	No sample	No sample
LT9	<dl	0.14 ± 0.04	<dl	0.01 ± 0.002	<dl	<dl	<dl	0.03 ± 0.01	<loq	0.12 ± 0.05
LT10	<dl	0.18 ± 0.02	<dl	0.04 ± 0.01	<dl	0.002 ± 0.002	<dl	0.04 ± 0.01	<loq	0.01 ± 0.01

<dl below detection limit of the assay

Table 6.2: Oestrogenic activity in Letsitele sediment samples using the YES assay and the T47D-KBluc reporter gene assay

Site	Sediment: EEq (ng/kg)									
	July 2011		November 2011		August 2012		November 2012		February 2013	
	YES	T47D-KBluc	YES	T47D-KBluc	YES	T47D-KBluc	YES	T47D-KBluc	YES	T47D-KBluc
LT1	<dl	<dl	<dl	0.71 ± 0.30	No sample	No sample	No sample	No sample	No sample	No sample
LT2	<dl	14.28 ± 2.58	<dl	8.06 ± 3.04	<dl	1.15 ± 0.32	<dl	1.86 ± 1.90	<dl	14.68 ± 1.14 *
LT3	<dl	2.04 ± 0.57	<dl	3.2 ± 1.2	No sample	No sample	No sample	No sample	<dl	0.10 ± 0.02
LT4	No sample	No sample	No sample	No sample	<dl	<dl	<dl	1.26 ± 0.93	No sample	No sample
LT6	No sample	No sample	No sample	No sample	No sample	No sample	No sample	No sample	No sample	No sample
LT7	<dl	<dl	<dl	1.83 ± 0.49	No sample	No sample	No sample	No sample	No sample	No sample
LT8	<dl	0.71 ± 0.14	<dl	1.36 ± 0.38	No sample	No sample	No sample	No sample	No sample	No sample
LT9	<dl	1.22 ± 0.32	<dl	0.68 ± 0.22	<dl	12.61 ± 0.93	<dl	10.23 ± 6.12	<dl	<dl
LT10	<dl	1.08 ± 0.30	No sample	No sample	No sample	No sample	No sample	No sample	No sample	No sample

<dl below detection limit of the assay; * cytotoxicity

Table 6.3: Oestrogenic activity in Lomati water samples using the YES assay and the T47D-KBluc reporter gene assay

Site	Water: EEq (ng/L)							
	June 2012		September 2012		December 2012		March 2013	
	YES	T47D-KBluc	YES	T47D-KBluc	YES	T47D-KBluc	YES	T47D-KBluc
NK 1	<dl	0.03 ± 0.004	<dl	0.005 ± 0.003	<dl	<dl	<dl	0.20 ± 0.09
NK2	<dl	0.07 ± 0.01	<dl	0.08 ± 0.06	<dl	0.28 ± 0.08	<dl	0.18 ± 0.06
NK3	<dl	0.08 ± 0.02 *	<dl	0.05 ± 0.02 *	<dl	0.24 ± 0.02	<dl	0.41 ± 0.04
NK3 tap	<dl	<dl *	<dl	<dl	<dl	0.22 ± 0.18	<dl	<dl
NK4	No sample	No sample	<dl	0.04 ± 0.02	<dl	0.37 ± 0.09 *	No sample	No sample
NK5	<dl	0.21 ± 0.04	<dl	0.02 ± 0.01	<dl	0.37 ± 0.15 *	<dl	0.35 ± 0.11 *
NK6	<dl	0.14 ± 0.07	<dl	0.09 ± 0.05 *	<dl	0.24 ± 0.06	<dl	0.42 ± 0.07 *

<dl below detection limit of the assay; * cytotoxicity

Table 6.4: Oestrogenic activity in Lomati sediment samples using the YES assay and the T47D-KBluc reporter gene assay

Site	Sediment: EEq (ng/kg)							
	June 2012		September 2012		December 2012		March 2013	
	YES	T47D-KBluc	YES	T47D-KBluc	YES	T47D-KBluc	YES	T47D-KBluc
NK 1	<dl	<dl	<dl	<dl	<dl	1.12 ± 1.13	<dl	0.73 ± 0.12
NK2	<dl	0.41 ± 0.02	<dl	7.43 ± 2.08	<dl	7.78 ± 0.71	<dl	1.56 ± 0.64
NK3	<dl	0.61 ± 0.18	<loq	65.04 ± 33.81	<dl	3.12 ± 0.48	<dl	4.97 ± 0.60
NK3 tap	No sample	No sample	No sample	No sample	No sample	No sample	No sample	No sample
NK4	No sample	No sample	<dl	15.92 ± 1.76	<dl	<dl	<dl	14.01 ± 1.43 *
NK5	<dl	0.30 ± 0.11	<dl	2.18 ± 0.57	<dl	0.80 ± 0.18	<dl	0.77 ± 0.15
NK6	<dl	1.91 ± 1.30	<loq	31.43 ± 13.72	<dl	12.17 ± 9.37	<dl	26.90 ± 0.86

<dl below detection limit of the assay; <loq below level of quantification of the assay; * cytotoxicity

Table 6.5: Oestrogenic activity in the Vals and Renoster water samples using the YES assay and the T47D-KBluc reporter gene assay

Site	Water: EEq (ng/L)							
	July 2012		October 2012		January 2013		April 2013	
	YES	T47D-KBluc	YES	T47D-KBluc	YES	T47D-KBluc	YES	T47D-KBluc
RN1	<dl	0.04 ± 0.006	<dl	0.11 ± 0.01	0.13 ± 0.03	0.56 ± 0.19 *	<dl	No sample
RN3	<dl	0.002 ± 0.000	<dl	0.08 ± 0.03	0.08 ± 0.01	0.33 ± 0.07 *	<dl	0.12 ± 0.02 *
RN4	<dl	0.01 ± 0.002	<loq	0.88 ± 0.47 *	0.50 ± 0.13	0.44 ± 0.13	<dl	0.19 ± 0.04 *
VL1	<loq	0.07 ± 0.02	<dl	0.16 ± 0.05 *	0.38 ± 0.08	0.03 ± 0.007 *	<dl	0.17 ± 0.04 *
VL2	2.13 ± 0.28 *	1.99 ± 0.22 *	<dl	0.69 ± 0.17 *	1.29 ± 0.12	6.78 ± 2.28 *	<loq	3.03 ± 0.41 *
VL3	<dl	<dl *	<loq	0.31 ± 0.07 *	0.21 ± 0.04	1.73 ± 0.37 *	<dl *	0.42 ± 0.29 *

<dl below detection limit of the assay; <loq below level of quantification of the assay; * cytotoxicity

Table 6.6: Oestrogenic activity in Vals and Renoster sediment samples using the YES assay and the T47D-KBluc reporter gene assay

Site	Sediment: EEq (ng/kg)							
	July 2012		October 2012		January 2013		April 2013	
	YES	T47D-KBluc	YES	T47D-KBluc	YES	T47D-KBluc	YES	T47D-KBluc
RN1	<dl	1.12 ± 0.80	<dl	0.80 ± 0.70	<loq	1.32 ± 0.21	<dl	5.16 ± 0.72
RN3	<dl	<dl	No sample	No sample	<loq	142.71 ± 48.54	<dl	3.69 ± 0.34
RN4	<dl	3.28 ± 3.99	<dl	0.91 ± 0.13	<dl	2.94 ± 0.33	36.02 ± 2.89	6.62 ± 0.90
VL1	<dl	0.18 ± 0.07	<dl	1.90 ± 1.68	<dl	1.47 ± 0.33	<dl *	13.88 ± 5.42 *
VL2	3.41 ± 0.42	6.65 ± 1.39	175.65 ± 11.85	170.42 ± 77.10 *	40.89 ± 1.50	2.64 ± 0.56	128.05 ± 17.50	129.05 ± 38.80 *
VL3	<dl	0.21 ± 0.06	<dl	<dl	18.13 ± 3.56	3.22 ± 1.61	90.80 ± 12.84	254.95 ± 69.20 *

<dl below detection limit of the assay; <loq below level of quantification of the assay; * cytotoxicity

Table 6.7: Oestrogenic activity in additional water samples using the YES assay and the T47D-KBluc reporter gene assay

Study Area	Site	Water: EEq (ng/L)	
		YES	T47D-KBluc
Letsitele	BM	<dl	0.01 ± 0.002
	SL	0.35 ± 0.04	0.004 ± 0.002
	FM	<dl	0.004 ± 0.0009
Vals & Renoster	KSD	<dl	0.009 ± 0.003
	KOP	0.28 ± 0.08	0.003 ± 0.0005
	VJK	<dl	0.002 ± 0.0006
Lomati	GW	<dl	0.007 ± 0.0004
	KC	<dl	0.006 ± 0.003
	RH	<dl	0.006 ± 0.001

<dl below detection limit of the assay

6.4 DISCUSSION AND CONCLUSIONS

Limitations of *in vitro* assays include the inability to detect compounds that are pro-oestrogens (compounds that are converted to oestrogens *in vivo*) or to conclusively identify the causative chemical without chemical analysis (Leusch *et al.*, 2010). However, they detect chemicals based on their biological activity and determine the total oestrogenic content of a given sample (Leusch *et al.*, 2010). Significant responses in an *in vitro* bioassay could be used as an indicator for further investigation using *in vivo* test models, and/or identification of the active chemical. A negative response would suggest that further investigations might not be warranted. Bioassays therefore play a major role as initial screening tools for environmental samples.

In this study oestrogenic activity was detected in water and sediment samples from all three agricultural areas. In general, sediment samples had higher EEqs compared to the water samples which may indicate bioaccumulation of oestrogenic compounds in the sediment.

Five of the water samples from the Viljoenskroon study area (RN4-Oct 12; VL2-Jul 12; VL2-Jan 13; VL2-Apr 13; VL3-Jan 13) were above the recommended trigger value of 0.7 ng/L EEq in drinking water that was proposed by Genthe *et al.* (2010). Cytotoxicity was also detected at the abovementioned sites. Further investigation of these sites is therefore recommended. A study of the area surrounding the sites and the data from the chemical analysis of the water samples might give some valuable information to identify possible sources of oestrogenic contamination at these sites.

It should be kept in mind that water and sediment samples consist of a complex mixture of chemicals with possible (anti)-androgenic and (anti)-oestrogenic activity, as well as other chemicals not measured, that could affect the outcome of the assay. It should therefore be noted that EEq's are only rough estimates, as the complexity of the sample, pH, extraction procedure and the nature of the assay (i.e. a biological system) might all have an influence on the results and may possibly lead to an under-estimation of the results or even to false negatives.

6.5 RECOMMENDATION

Further investigation is required to identify the source of oestrogenic contamination of the VL2 sample site. High levels of oestrogenic activity were measured for all sampling periods with the highest activity measured in January 2013. No direct causative link could be established between the chemical analysis and the activity in the samples collected from this area. It should be kept in mind that there may be other potential sources other than agricultural pesticides that could contribute to the total activity seen in the samples (e.g. VL2 was downstream of the town of Kroonstad and therefore likely receives sewage and industrial effluent which may have contributed to the relatively high values recorded at this site). It is therefore recommended that other potential chemical or sources of oestrogenic activity be investigated.

While the bioassays used in this study can measure oestrogenic activity in environmental samples, more work is needed to identify trigger values which will be especially important when determining levels of associated risk to human health and the environment.

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CHAPTER 7: PRINCIPLE COMPONENT ANALYSIS

by

Rialet Pieters

Unit for Environmental Sciences and Management; North-West University; Potchefstroom, 2520

7.1 INTRODUCTION

Multivariate statistical analysis, specifically principal component analysis (PCA), was done on those samples for which entire sets of chemistry data as well as bioassay results were available. For this reason sediment samples were not included because there were no levels for inorganic chemicals available.

PCA is used to search for similarities and differences in pollutant patterns in a wide range of environmental media and then infer source links. This method reduces several variables to a few underlying descriptive dimensions that can be used to explain patterns within a set of observed values (Alcock *et al.*, 2002). These underlying descriptive dimensions are uncorrelated linear combinations of the original variables that explain decreasing amounts of the total variation—the first linear combination or component explains the maximum percentage of variance, and so on (Howel, 2007). These percentages of variance are referred to as Eigen values.

The importance of each pollutant in determining the position of individual samples and clusters of samples along the principal component axes are partially explained by the principal components loadings plot. The pollutant with the highest contributions to the differences observed between samples and among clusters are located farthest from the origin of the axes, The greater the distance along a given principal component axis, the more a pollutant contributes to the variation accounted for along the axis. A negative or positive position along the axis indicates whether a congener is negatively or positively correlated with the principal component. A sample positioned along a positive principal component axis contains low concentration of pollutants with negative loadings, and higher concentrations of pollutants with positive loadings. A sample positioned along a negative principal components axis contains the opposite relationship. As a result the position of samples or clusters indicates the differences in the composition of the pollutants (Wenning *et al.*, 1992).

7.2 MATERIAL AND METHODS

The motivation for the PCA was to attempt to answer whether there is a relationship between the observed bioassay responses and the organic and inorganic compounds which had quantified levels. Not all the quantified inorganic compounds had been included in the analysis. The following ones were selected because of their known or suspected ability to interfere with hormonal systems: fluoride (F^-),

nitrite (NO_2^-), nitrate (NO_3^-), bromide (Br^-), copper (Cu), manganese (Mn) and selenium (Se). The quantified organic compounds included the following: alachlor (Alachl), atrazine (Atrz) diphenylamine (Dpham), imidacloprid (Imclpd), imizalil (Imzlil), and propiconazole (Ppczole), simazine (Smze), and thiabendazole (Thbdzl). The particular combination of organic compounds for which quantified concentrations were available varied between the three main geographical regions. For Letsitele they were: Dpham, Imzlil, Thbdzl, Imclpd, and Ppczole. At Lomati the list included: Dpham, Imzlil, Thbdzl, Imclpd and Ppczole and at Vals and Renoster Rivers catchment they were: Alachl, Atrz, Imclpd, Smzne, and Tbthz.

The bioassay results—or mechanism of action—comprised of androgen activation (AndAct) and/or androgen inhibition (AndInh) and oestrogen activation was quantified using two assays, the yeast screen (YES) and the reporter gene cell line. The latter data is labelled OestAct on the graphs. If an assay's results did not have a single quantified level, that particular assay response was not included in the PCA. This led to AndAct, AndInh and OesAct for Letsitele and Lomati, but AndInh, YES and OesAct for Vals and Renoster Rivers.

Samples were coded according to the catchment, (e.g. RN for Renoster River or LT for Letsitele) together with the particular site number as well as the month and year of sampling. A "W" or "S" was added to the code to distinguish between sediment (S) and water (W) samples but the sediment samples never made it to the PCA as explained above. A sample designation "VL2W01_13" therefore indicates to the water sample from the second site in the Vals River (VL) that was collected in January 2013 and "LT9W11_12" should be interpreted as the water sample from the ninth site in the Letsitele River that was collected in November 2012.

In order to minimise the effects associated with either orders of magnitude differences in concentrations across samples, and to incorporate samples measured in different units, individual concentrations were expressed as a proportion of the sum of all other pollutants concentrations reported for a particular sample (Alcock *et al.*, 2002; Masunaga *et al.*, 2003). This treatment reflects the relative size of component parts, and the resulting proportions are known as the pollutant profile or "fingerprint" of a sample. Such data is often referred to as compositional. Howel (2007) motivated for the use of log-ratio transformations on compositional data prior to using standard multivariate analysis rather than the compositional data because the linear dependence of proportions in compositional data leads to an absence of an interpretable covariance structure. This causes the apparent strength and direction of the association between two proportions to appear to depend on the number of elements in the total composition and also to differ for different subsets of element. Since compositional data attempts to describe the relative magnitudes of pollutants the ratios of proportions should remain the same independent of the number of pollutants in a composition. Because this property is absent from compositional data, standard statistical methods cannot be used. Aitchison (1986) proved that this could be rectified when dealing with either of the following two functions of log ratios of proportions rather than with the compositional data. If d pollutants are measured in n samples so that p_{ij} is the proportion of pollutant i (i = 1, 2, ..., d) in sample j(j = 1, 2, ..., n), then the two most common choices of sets of log-ratios are:

1. The *d*-1 log-ratios obtained by dividing the proportions of each pollutant by the proportions of one of the pollutants xlog(p_{ij}/p_{ij}).
2. The *d* log-ratios obtained by dividing each proportion by the geometric mean across the sample log($p_{ij}/g(p_j)$) where $g(p_j) = (p_{1j}, p_{2j}, ..., p_{dj})^{1/d}$. These are referred to as "centred log-ratios" (Howel, 2007).

Howel (2007) motivated that the principal components for compositional data should be derived from the covariance matrix of the centred log-ratios of proportions (equation no 2), and not the covariance or correlation matrix of the proportions. Therefore, the levels of the compounds and the assay results were included in the PCA were first treated as described in equation no 2 above. This led to the substitution of all "below limit of detection or quantification" (<LOD or <LOQ) values with half the value of the smallest value reported for that geographical area for that particular compound. Substituting with zero renders causes that logs cannot be calculated and since no LOD or LOQ was reported for the organic chemical data, this route was followed. CANOCO software was used to execute the PCA on the already transformed data.

7.3 RESULTS AND DISCUSSION

7.3.1 Letsitele catchment:

Factor 1 of catchment's PCA explained 50% of the variance in the data, followed by 17.7%, 14.3% and 6% respectively for factors 2, 3, and 4. Factor 1 consisted of the contrast between organic compounds thiabendazole, imizalil and imidacloprid on the positive side with oestrogen activity (as measured by the reporter gene bioassay) and propiconazole having loaded the most on the negative side of the factor (Figure 7.1). The samples with the highest positive score were two samples from the August 2012 sampling event—and one from the February 2013 event. LT10W02_13, LT9W08_12 and LT10W08_12 had the highest factor scores (Figure 7.1). These sites appeared on the opposite end of the factor where the sites with the biggest negative scores positioned: LT4W02_13 and LT3W11_12 (Figure 7.1). It seems as even though propiconazole has oestrogen inhibitive characteristics, of all the compounds and chemicals included in this PCA, it was the one most likely to be responsible for the oestrogen receptor (ER) activation in the reporter gene bioassay for samples LT4W02_13 and LT3W11_12. This is contradicting expectations as two of the three compounds with high loadings for factor 1 (i.e. not associated with ER activation), thiabendazole and imizalil are known to exhibit at least weak ER activation (Hurst and Sheahan, 2003; Trosken, 2004).

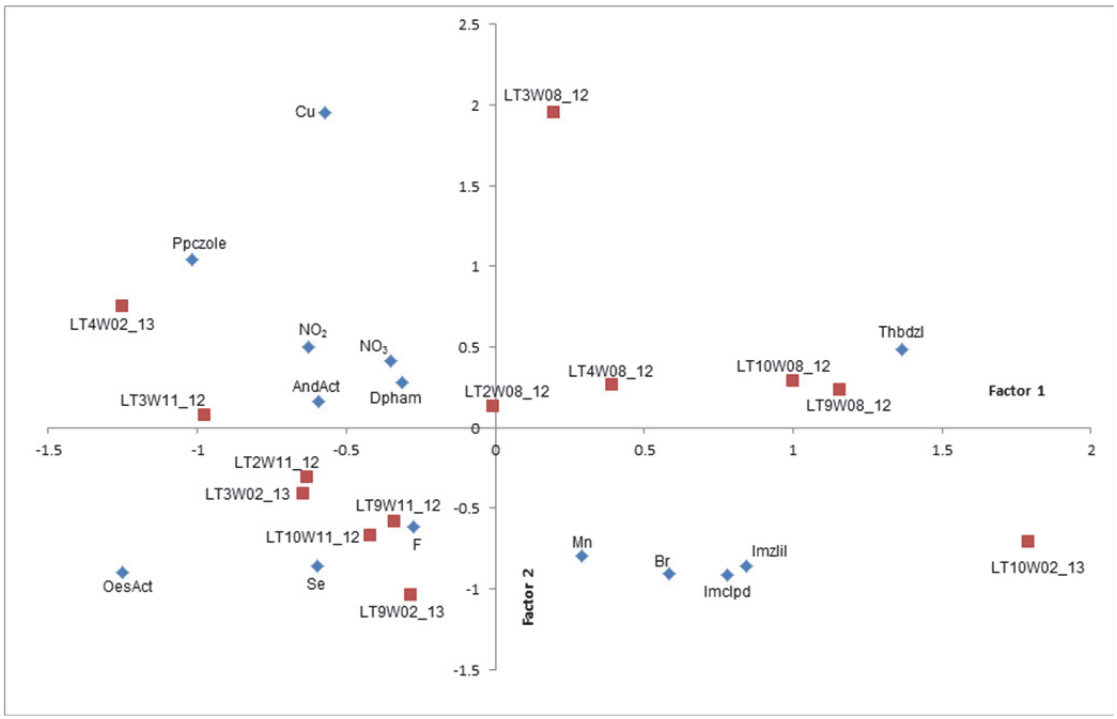

Figure 7.1: Biplot of factor 1 and factor 2 for the Letsitele catchment with both the loadings (compounds) and the scores (samples)

Both factors 2 and 3 have organic and inorganic compounds contributing to them. Factor 2 has copper and propiconazole loading with high values on the positive side, with oestrogen activity loading on the negative side of the same factor together with bromide and imidacloprid (Figure 7.1). Again, contradicting pieces of information coming to the fore: bromide has been found to be an oestrogen inhibitor (Wu *et al.*, 2014) but for this factor it clustered with ER activation (OestAct). Imidacloprid also loaded with the ER activation on this factor, while it loaded opposite to ER activation in factor 1. Furthermore, copper clustered with propiconazole and whereas Cu does not actively bind to the ER it has the ability to potentiate the oestradiol-induced response of endogenous hormones by modulating the estrogenic activity of endogenous hormones in a dose-response manner (Denier *et al.*, 2009). However, Cu, when complexing with compounds like 17β-oestradiol, its artificial equivalent, 17α-ethynyloestradiol and octylphenol decreases these compounds' ability to bind to the ER (Park *et al.*, 2008). Sample LT9W02_13 had the lowest score for factor 2 (Figure 7.1), indicating that of the compounds included in this PCA, the ER binding activity observed for this sample was most likely due to bromide and imidacloprid.

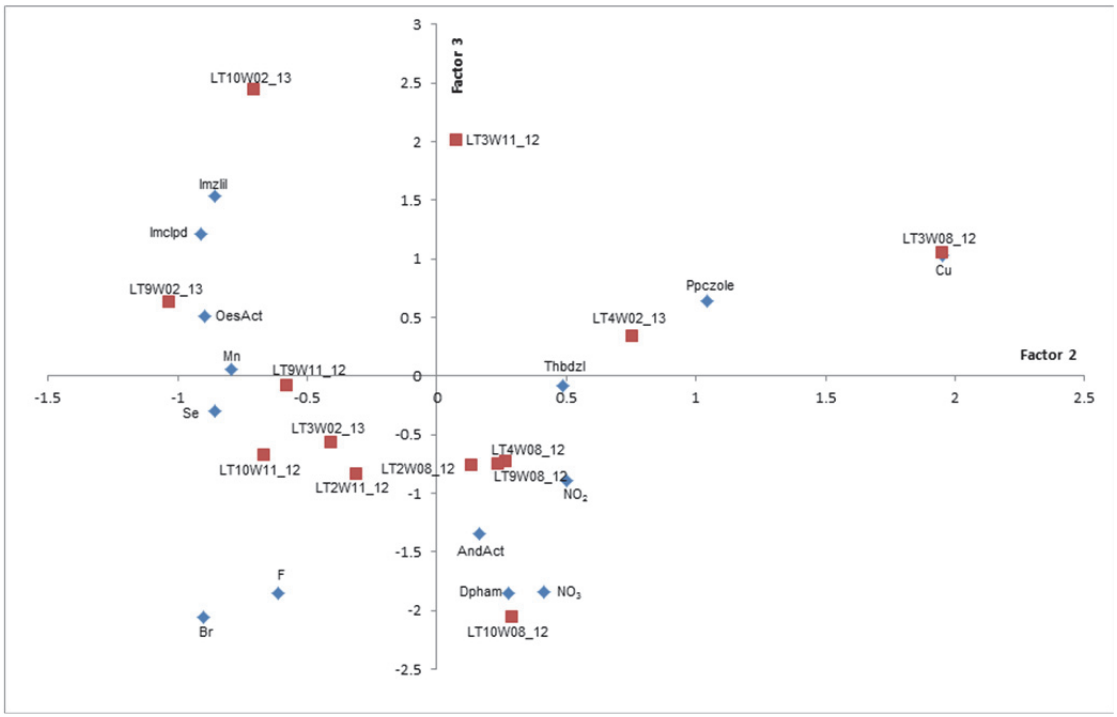

Figure 7.2: Biplot of factor 2 and factor 3 for the Letsitele catchment with both the loadings (compounds) and the scores (samples)

Factor 3 consists of a contrast between imizalil, imidacloprid and Cu on the positive side and Br, diphenylamine, F and NO_3^- loading on the negative side. Samples LT10W02_13 and LT3W11_12 had high positive factors scores, with a single sample on the negative side of factor 3, LT10W08_12 (Figure 7.2). Factor 3 seems to contrast organic compounds (positive side) to the more inorganic compounds (negative side). The selection of compounds that loaded onto the various ends of the factor does not seem to share obvious oestrogenic/androgenic characteristics which would explain their clustering: imizalil is oestrogenic active, and anti-androgenic (Hurst and Sheahan, 2003; Trosken, 2004); the behaviour of imidacloprid is uncertain and Cu can be agonistic as well as antagonistic depending the circumstances (Denier *et al.*, 2009; Park *et al.*, 2008). On the opposite side of the same factor: Br and diphenylamine are known ER inhibitors (Wu *et al.*, 2014; Ohta *et al.*, 2015); F when substituted into oestradiol can inhibit enzymes in the steroidogenesis process (Deluca *et al.*, 2006) and NO_3^- activates oestrogen receptor α (Veselik *et al.*, 2008).

7.3.2 Renoster & Vals Rivers catchment:

The first four factors explained approximately the same percentage of variance (82%) as the first four factors of the Letsitele catchment 81%, however, for the RenosterVals catchment factor 1 explained only 35% of the variance. As for the Letsitele catchment, the factor consisted of a contrast between clusters of organic compounds only, but a different collection of compounds (Figure 7.3). The compound with the highest positive score was simazine which clustered with the ER activation (OesAct). The largest negative loadings were found for alachlor and imidacloprid which grouped with the effect of androgen receptor (AR) inhibition

(Figure 7.3). The samples with the highest positive scores for factor 1 were three samples from the Vals River (VL2W01_13, VL2W04_13, and VL3W04_13). It was samples from the Renoster River which had the lowest scores for factor 1: RN3W04_13, RN1W04_13, and RN3W07_1 (Figure 7.3). Judging endocrine effects observed for this catchment merely referring to factor 1 it seems as if the Vals River was exposed to ER active compounds and the Renoster River to androgen inhibitive compounds. The clustering of simazine with Oestact was unexpected because there seem to be no clear evidence of simazine capable of ER activation in literature (Connor *et al.*, 1989; Kojima *et al.*, 2004). Furthermore, alachlor with a known weak oestrogenic activation effect (Klotz, *et al.*, 1996) loaded with the androgen inhibition effect (AndInh) and the undecided imidachloprid.

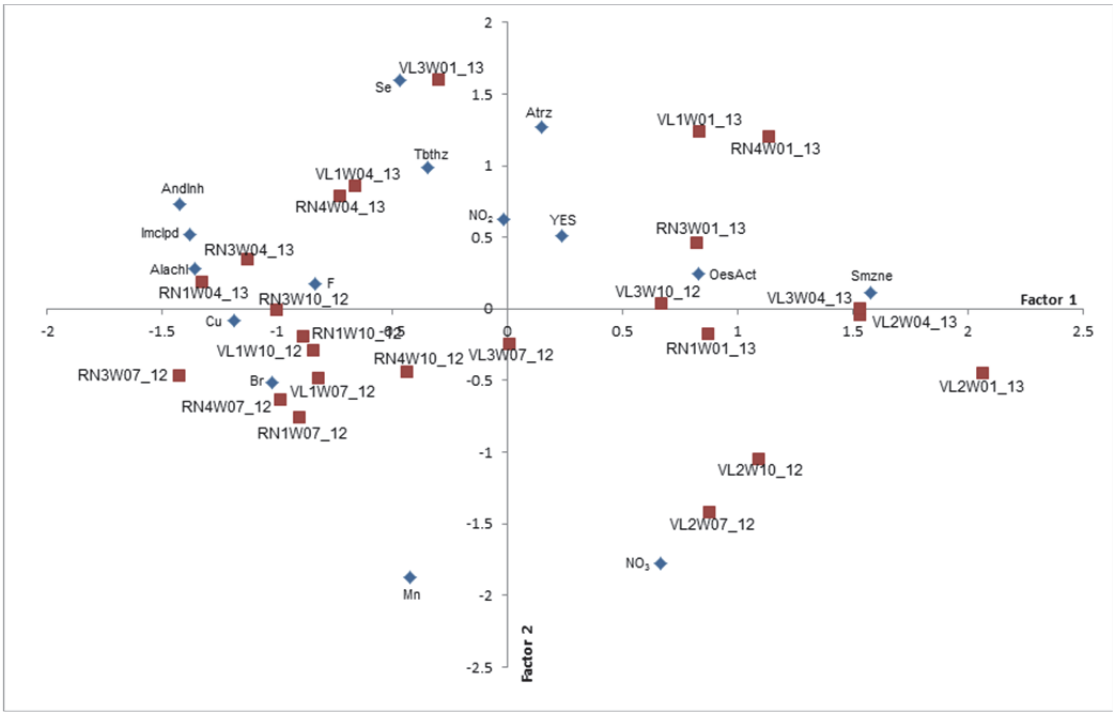

Figure 7.3: Biplot of factor 1 and factor 2 for the Vals and Renoster Rivers catchment with both the loadings (compounds) and the scores (samples)

Factor 2 explained 18.6% of the variance in the data and was formed by a contrast between selenium, atrazine, and terbuthylazine with strong positive loadings and NO_3^- and manganese on the negative side of the factor (Figure 7.3). Samples with scores for this factor were mostly from the Vals River with one exception. Two samples from the January 2013 event, VL3W01_13, and VL1W01_13, and one for the Renoster River, same sampling event, RN4W01_13 had high factor scores for factor 2 (Figure 7.3). There was only one sample which had a high negative score for this factor, also from the Vals River, VL2W07_12 (Figure 7.3). The three compounds with the highest loadings for factor 2 do not have any clear ER or AR activity that could be found in literature. Selenium is associated with thyroid related problems (Balázs and Rácz, 2013; Beckett and Arthur, 2005); although atrazine creates reproductive problems it is not via binding to the AR or ER and activating or inhibiting it under environmental conditions (Eldridge *et al.*, 2008) and terbuthylazine's endocrine disruptive activity is also via another mechanism of action

(Sanderson *et al.*, 2000). This could mean that the samples with the highest scores for factor 2 might have contained compounds - selenium, atrazine and terbuthylazine - with ED activities other than the mechanism of action the bioassays this study used can measure. The two compounds with loadings on the negative side of factor 2 both have ED activity of which only NO_3^- has ER activation capabilities and only for α-ER. Manganese dioxide can remove oestrogen from solutions (Jiang *et al.*, 2010).

Factor 3 explained 16% of the variance and consisted of mainly two compounds: fluorine on the positive side and the OestAct on the negative side (Figure 7.4). Two samples VL3W07_12 and VL1W01_13 had high positive scores for factor 3 and sample RN4W10_12 had a high negative score. (Figure 7.4)

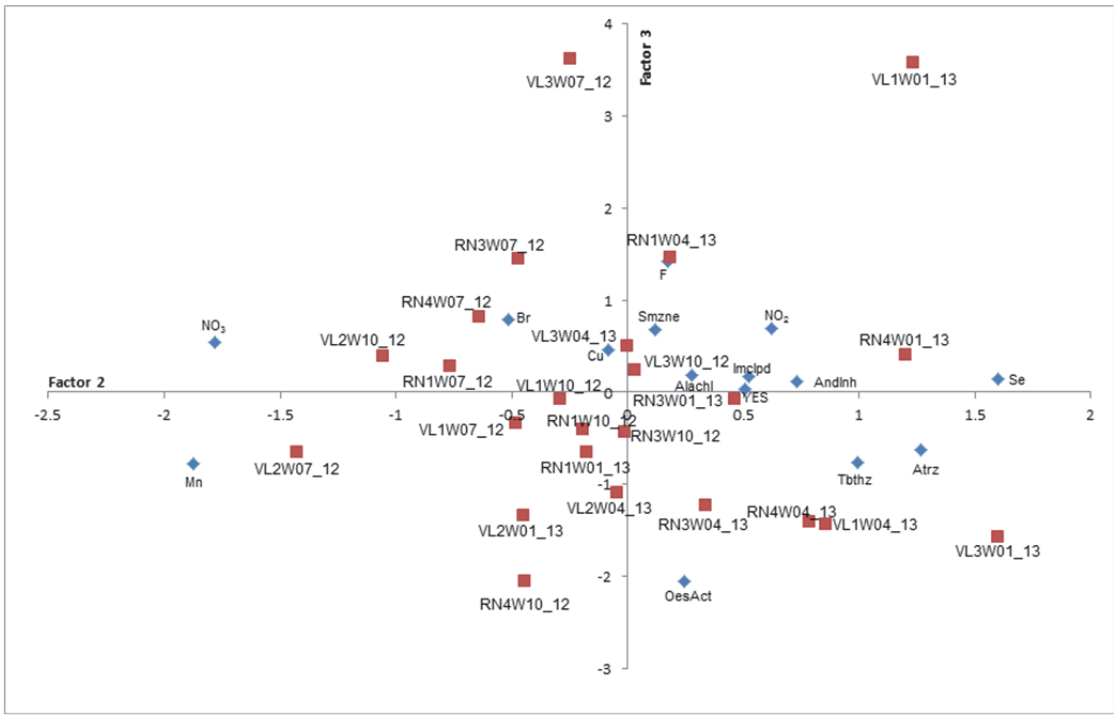

Figure 7.4: Biplot of factor 2 and factor 3 for the Vals and Renoster Rivers catchment with both the loadings (compounds) and the scores (samples)

7.3.3 Lomati catchment:

The Eigenvalues indicated that factor 1 explained 49% of the variance while 37.4% was explained by the next three factors. The pollutants which loaded onto the positive side of factor 1 were carbofuran, atrazine, terbuthylazine, and imidacloprid (Figure 7.5), all of which have reported ED activity except for imidacloprid (Matisová and Hrouzková, 2012). As for atrazine and terbuthulazine, carbofuran's ED activity is via mechanisms of action other than binding to the ER (Nishihara *et al.*, 2000). The pollutants and effects that loaded onto the negative side of factor 1 were inhibition of the androgen receptor (AndInh) as well as its activation (AndAct), bromine, nitrate, manganese and iron (Figure 7.5). Of the three catchments investigated in this study, it was only one in which (anti-)androgen activity contributed to any of the factors, and here it had loadings for factor 1, indicating their important role. It therefore seems

that of all the compounds included in the PCA, those listed above to have negative loadings for factor 2, seem to contribute to (anti-)androgen activity. This, however, could not be corroborated from literature. The samples which had high positive scores for factor 1 were all from the same sampling event, September 2012: NK4W09_12, NK6W09_12, NK3Tap09_12, and NK3W09_12 while the samples with the most negative factor scores were NK3W06_12, NK5W06_12, NK4W06_12, NK6W06_12, and NK2W06_12, all from the June 2012 sampling event (Figure 7.5). The latter list of samples contains the ones most likely to have caused (anti-) androgen activity.

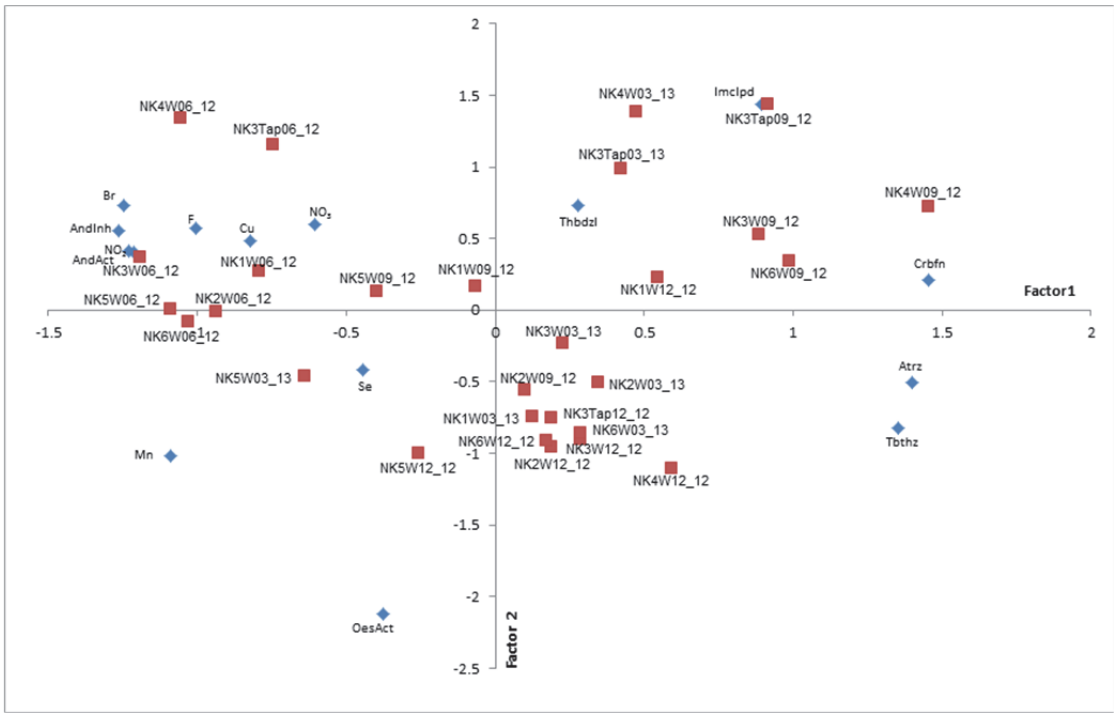

Figure 7.5: Biplot of factor 1 and factor 2 for the Lomati catchment with both the loadings (compounds) and the scores (samples)

Factor 2 was contributed to by imidacloprid on the positive side and OestAct, manganese and terbuthylazine had loadings on the negative side (Figure 7.5). From this it seems that manganese and terbuthylazine could have contributed to AR activation of the samples with the most negative scores for factor 2, however, there were no samples with noteworthy negative scores for factor 2. The samples with high positive scores were NK6W03_13 and NK2W03_13 both from the March 2013 sampling event (Figure 7.5).

Factor 3 was created by a contrast between imidacloprid, nitrite and OestAct on the positive side and nitrate and atrazine on the negative side (Figure 7.6). The samples that had high scores for factor 3 were also from March 2013: NK6W03_13 and NK2W03_13, while one sample from September 2012, NK1W09_12 had a noteworthy score for factor 3 (Figure 7.6).

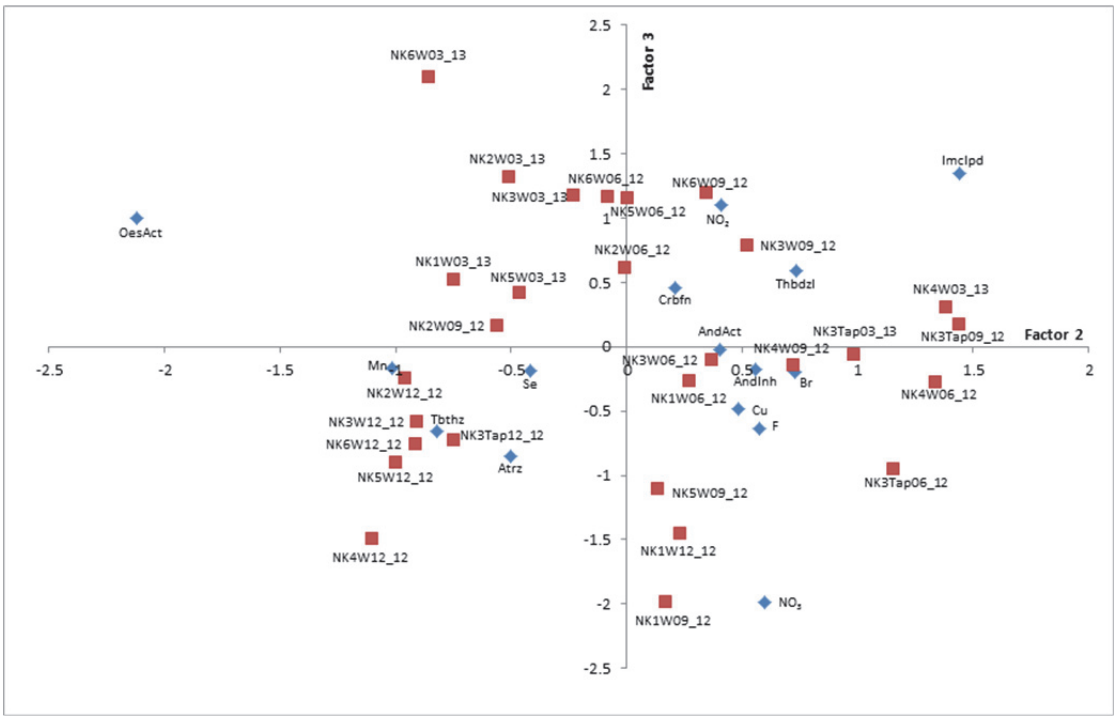

Figure 7.6: Biplot of factor 2 and factor 3 for the Lomati catchment with both the loadings (compounds) and the scores (samples)

7.4 CONCLUSION

PCA is an investigative statistical procedure which indicates to inherent relationships between data points included in the analysis. Mostly the analysis for the three catchments showed relationships between compounds and oestrogenic and/or androgenic receptor binding that could not be supported by what is found in literature. Although the organic and inorganic compounds that were included in the PCA all have endocrine disruptive characteristics, the mechanism of action that was measured in this study and included in the PCA, is binding to the respective oestrogen and androgen receptors and not many of the compounds included in the PCA had proven ER and/or AR binding capabilities. This could be corroborated by composition of the compounds loading on the various factors: whenever an effect loaded high on either the negative or positive side of a factor, the compounds also loading with that effect do not mainly affect its ED capabilities through binding to the cellular receptor.

The two most interesting relationships that crystallised from the PCAs are:

1 The PCA of the Vals and Renoster Rivers catchments separated the two rivers, indicating towards different pollutant profiles.

2 In the two catchments where atrazine and terbuthylazine were used, they contributed with similar loadings to the same factor, cf factor 1 for the Lomati catchment and F2 for the Vals and Renoster River catchment.

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CHAPTER 8: ANIMAL HEALTH ASSESSMENT

by

J.A. Meyer

PO Box 2312, Faerie Glen, Pretoria

8.1 INTRODUCTION

As the title of the project suggests the primary investigation objective is to evaluate the impact of agricultural chemicals on water resources with both potential animal and human health effects assessed. Environmental and human health effects are presented in separate sections of this report, and whilst the animal assessment is presented here relevant cross-links applicable are highlighted.

As a point of departure it should be noted that the types of effects agricultural chemicals may have on animal health are too varied and complex to be presented here, but a summary thereof was presented as a deliverable for this project and the reader is referred to the deliverables for further detail. By way of introduction it may be noted that the types of effects relevant are not simply direct adverse health effects. When viewed in light of the objective of many Acts to protect and provide a sustainable economic environment, the types of effects relevant extend to include those effects which may have a negative impact on the production parameters required for successful animal production systems (Casey and Meyer, 2006). It is beyond the scope of this chapter to detail these systems, but it should be appreciated that they differ significantly between rural communal subsistence systems and intensive commercial production systems. The types of effects relevant are thus a function of the system relevant itself and not just the animal or water quality constituent in question. The types of effects may be broadly categorized by the following norms:

- Animal Health Norms:
    - Toxicological Effects, both direct and indirect consequences thereof:
        - For example, chronic dental and skeletal fluorosis which can affect health directly by the toxicological actions of fluoride on teeth and bones, and indirectly by reducing production by lowering water and feed intakes by causing pain when drinking water and walking to graze or forage.
    - Palatability Effects:
        - For example, trace elements (such as zinc, manganese and iron) and macro elements (such as sulphate and Total Dissolved Solids), which can lower intakes of water and thus food, effectively reducing growth and production parameters.
    - Induced Deficiencies:
        - The presence of some constituents may not have direct adverse effects associated with an excessive exposure, but due to mineral-

mineral interactions may lower the availability of other elements. These may be complex involving several different elements and can result in an increased dietary requirement for essential minerals. Examples include interactions between copper, molybdenum and sulphate.

- Other health effects, including Carcinogenic Effects and Endocrine Disruptive Effects:
  - These may be relevant in some instances and not in others, for example, animals such as broilers grown for slaughter at 35 days may not be significantly affected by carcinogenic effects, but breeding stock required to produce genetic material may be.

- Water Distribution Effects:
  - Constituents affecting water pressure can reduce intakes of both water and thus feed (usually biofilm and scaling).
  - Constituents entering the drinking water by corrosion may impart a negative taste to the water and thus lower intakes.
- Product Quality Norms:
  - These may involve product quality from a consumer safety and health perspective. In some instances the constituent may result in the animal product being unfit for use by a consumer without significantly affecting the animal itself (e.g. mercury and lead accumulation).
  - These may also have a direct adverse effect on product quality, for example eggshell thickness.
- Environmental Norms:
  - These may relate to the wastewater composition produced from confined animal feeding operations and fitness for beneficial reuse thereof by impacting several aspects, ranging from ammonia to heavy metal content.
  - These may also affect the movement of free-ranging animals and subsequent impact on the local environment, for example sacrifice zones and impacts on herbaceous communities in wildlife enterprises.

Adding to the complexity of performing an assessment, some constituents may affect multiple norms, for example fluoride, which may have both direct toxicological pathways and endocrine disruptive effects. In the case of nitrate, the effects may be direct toxicological (methemoglobinemia), endocrine disruptive and also water distribution, by promoting the growth of algae and biofilm in drinker lines leading to lower water intakes and potential toxic algae concerns. It should be appreciated that the current existing 1996 South African Water Quality Guidelines for Agricultural Use (Livestock Watering Volume) are outdated in comparison to existing approaches in terms of animal health and in many instances, due to the strict biosecurity measures and high performance requirements, some commercial animal production systems may apply more conservative guideline target water quality ranges than others (and at times be stricter than domestic guidelines).

The current Water Research Commission Short-Term Research Project (WRC K8/1067) on developing Risk-Based Water Quality Guidelines should be consulted for more detailed background information, including the Phase 1 Report completed in 2008 (Development of SA Risk-based Water Quality Guidelines: Phase 1, Needs Assessment and Philosophy: available from the WRC).

As a member of the Global Water Research Coalition (GWRC) the Water Research Commission (WRC) of South Africa has been involved in a collaborative international water research alliance since 2002. Although the GWRC focus is predominantly on the urban water cycle, renewable resources also form part of the focus areas. Whilst this project deals with agricultural chemicals, many of these are noted in the literature and WRC reports as potential and recognized EDCs. Accordingly, the approach to EDCs may be argued as also being applicable to agricultural chemicals, both in terms of the assessment of effects in different users, and the challenges posed towards management thereof in water resources.

In a recent review on endocrine assays (GWRC, 2012) it was observed that many of the bioassays had been applied to patchily to wastewater and that further information is required for other waters, such as drinking water, groundwater and surface water. It is also noted that whilst it is clear that not all endpoints affected by EDCs are equally understood or researched, they are regarded as posing a serious threat to human and animal health. Despite it being stated that “It is still unclear whether the concentrations detected are sufficient to case significant endocrine disruption”, the review concluded that “Nevertheless, endocrine disruption is a potential serious threat to human life even at low concentrations, and it is important to understand the contribution from water and its relevance.”

This observation is fundamentally valid for agricultural chemicals as well. To illustrate this point further, in many instances the acceptable concentrations of a pesticide in drinking water was based on specific types of toxicological (dose-response curve derived) and carcinogenic (slope-factor derived) effects, whilst it is now recognized that for some EDC effects may also be a primary hazard. Thus, the current guideline value, in not accommodating EDC effects, thus cannot provide any meaningful hazard or risk statement following exposure. It is also noteworthy that the existing guidelines do not cater for multiple exposures to potentially hazardous constituents as a collective assessment. In most instances this is simply due to the complexities and uncertainties surrounding the vast array of possible interactions (refer to Meyer *et al*., 2014 for detailed a discussion).

These factors may also apply to inorganic elements such as nitrate, fluoride and bromide, which may to a greater or lesser extent, have some defined cause and effect relationships, but which also are accepted as having the potential to disrupt the endocrine system. The approach for EDCs may thus be argued to be relevant for many agricultural chemicals with sufficient motivation existing to adopt a precautionary principle approach.

The WRC developed a Manual on Endocrine Disrupting Chemical Management in Water Resources in a more user friendly format that would reach a wider target audience. The Manual takes the form of several Volumes, with the aims of

synthesizing available research, stimulating and assisting on-going research regarding multidisciplinary fields relevant to EDCs.

The Volumes of the EDC Manual also serve as an appropriate backdrop to agricultural chemicals and the on-going efforts to understand the significance of the role they play in water. These Volumes assist a wide target audience, including water supply agencies, water resource managers, workers in health and water related fields, educators and communities, and whilst not repeated here, technical detail on many of the methodologies used in the various Chapters of this report may be found therein.

8.2 METHODS AND CONSIDERATIONS

8.2.1 General approach adopted

As noted and described elsewhere in this report several different sample types were obtained from three key selected sites in order to investigate the occurrence and potential effects of agricultural chemicals. Site selection is detailed in previous deliverables submitted for this project and presented elsewhere in this report, with details of the locations and sampling point descriptions not repeated here. Simultaneously collection of other samples and different sample media was performed for a variety of pesticide and endocrine disrupting screens, which are also reported elsewhere.

It may be broadly stated that the purpose of obtaining the water samples was primarily threefold:

- To adequately describe baseline conditions pertaining to water quality with specific reference to organic and inorganic chemistry, and selected physico-chemical properties, in order to assist with the hazard and risk assessment procedures formulated by additional sampling techniques generally related to agricultural chemicals, pesticides and potential endocrine disrupting effects and other potential adverse effects;
- To perform an assessment of the fitness for use for recognized water uses, specifically livestock and domestic use;
- To compare the results obtained with other observations regarding the use of sentinel broiler specimens for exposure outcomes and adverse effect endpoints relating to inorganic agricultural chemicals; and
- To establish a monitoring platform.

Following the observations of the various sample testing results additional targeted assessments were conducted. These included obtaining various tissue samples from poultry specimens in rural and commercial systems and conducting tests for clinical biochemistry and histopathology.

Although the three sites served as the main sampling areas additional observations from relevant sites are also included. Notably these include assessments from

wastewaters generated by animal production systems in recognition of the relevant changes that occurred during the project lifetime to Schedules to Acts dealing with the environment and water. Finally, tissue observations from studies with key potentially hazardous agricultural chemical constituents in drinking water for animals are presented which demonstrate the significance and context of adverse effects and responses to mitigation efforts. This is presented in greater detail in the relevant sections of this Chapter.

8.2.2 Sample Collection and Analysis

8.2.2.1 Water and Wastewater

Samples were initially collected primarily from surface water supplies, with groundwater collected in the latter stages of the project. It should be noted that the site selection did not focus on groundwater as a primary resource, but more on the use of agricultural chemicals (notably pesticides and related crop production chemicals) in catchments. Accordingly, the three sites did not represent agricultural production activities for which groundwater represented the sole source.

For water samples, the inorganic chemical analysis was conducted by ICP-AES and ICP-MS techniques by the Institute for Soil Climate and Water at the Agricultural Research Council, Pretoria, using full quantitative and semi-quantitative procedures (ISCW, 2001 - AGRILASA). For the inorganic chemicals, full detail regarding the sampling methodology recommended is presented in a separate Water Research Commission Report: K8/999: “Sampling and Methods for the Analysis of Inorganic Endocrine Disrupting Chemicals” (April 2012). Laboratory prepared sample containers as prescribed in the standard methods were used with samples collected on site and delivered to the laboratories for pre-arranged processing.

Microbiological assessments were performed by the Food & Microbiological Section at the South African Bureau of Standards in accordance with SANS 241:2011 (SANS 2011).

The physico-chemical parameters were conducted by the Water Chemistry Section at the South African Bureau of Standards in accordance with SANS 241:2006 (SANS 2006).

The recommended sampling procedures appear in “Quality of Domestic Water Supplies, Volume 2”, and can be obtained from the Water Research Commission (QDWS, 1998). For wastewater samples (both solid and liquid fractions), the sampling and analytical methodology employed accords with the prescribed WRC Technical Reports as stipulated in the Government Notice No. 665 of 6 September 2013 (National Water Act. Act 36 of 1998) as noted in Section 1.10 (2) of the Schedule: “… the resulting sludge disposed of according to the requirements of any relevant law or regulation, including Guidelines for the Utilisation and Disposal of Wastewater Sludge, Volumes 1-5, Water Research Commission Reports TT 261/06, 262/06, 349/09, 350/09, 351/09, as amended from time to time.”

Further references may be obtained from the following sources:

- Standard Methods for the Examination of Water and Wastewater (1985). 16th Edition. American Public Health Association. Washington DC 20005. ISBN: 0-87553-131-8
- SANS 5221:2007 (Edit 4.3): Microbiological quality of water: General Test Methods.
- F.M. 5.4 W-B: Total coliforms and *Escherichia coli* in water – Defined Substrate Technology (Colilert) Method.
- SANS 241-1:2011 Edition 1. Bibliography. Standards. Pages 12-14. Annual book of ASTM standards and SANS Standard Test methods.
- Sampling and Methods for the analysis of Inorganic Endocrine Disrupting Chemicals: Water Research Commission Report K8/999 (Meyer *et al.*, 2013).
- WRC Project K5.1915: Volume 4 of the Manual on Endocrine Disrupting Chemicals: Monitoring and Assessment of EDCs (Meyer *et al.*, 2014).

8.2.2.2 Sentinel Sampling

Poultry specimens were collected from both the Letsitele site and the Komatipoort study area (Table 8.1). The Letsitele samples were obtained from households in the village and represented a wide variety of indigenous poultry breeds of various ages, but all mature specimens. The Komatipoort samples were obtained from a commercial production unit with open-sided houses using Ross 308 broilers. A second round of poultry specimens were obtained from the Komatipoort site following additional veterinary observations of feminization in cockerels (an EDC effect).

Additional observations using a porcine model are also presented as the results obtained provide further support for the methods adopted and main conclusions drawn.

The specific methods for the ICP-MS determination are presented in WRC Report K8/999 (Meyer *et al.*, 2013) but briefly involve a multi-element microwave assisted acid digestion of the tissues prior to analysis by the ICP-MS method also described in the WRC report referred to. Duplicates were prepared for each tissue type with the arithmetic mean of each duplicate used as the individual specimen value with pooled median values used as site observations. This technique is used for determining clinical biochemistry trends in investigating nutritional pathologies. Although this was not the emphasis of this investigation such interpretations may be performed such it be required. The goal of this method is total sample decomposition with acids. A benefit to this method is that the same ICP-MS apparatus is used for water, sediment and biological tissues with a significant benefit in reducing inter-laboratory variation. This is advantageous from a monitoring perspective.

Table 8.1: Details of sampling and analysis of biological tissues from poultry.

Biological Tissue Detail	
First Phase: A total of 10 specimens from each site were collected (mature from Letsitele and at day 35 in the production cycle for Komatipoort) and sacrificied by cervical dislocation. *Second Phase:* A total of 8 specimens from the Komatipoort site were obtained and sacrificied by cervical dislocation.	
Sample Type	**Test Performed**
Whole Blood	Collected from the Brachial Vein in lithium heparinized vacutainers (LH BD Vacutainer 4mL) from live bird. Tested for plasma thyroxine (Total T4). Submitted to: Veterinary Hormone Laboratory Faculty of Veterinary Science University of Pretoria
Tissues: Thyroid (bilateral) Thymus (bilateral - poultry) Bursa of Fabricius (poultry) Testis (male broilers)	**Histopathology:** Samples were immediately fixed in 10% buffered formalin and submitted to: Department of Paraclinical Sciences Faculty of Veterinary Science University of Pretoria
Tissues: Hepatic (whole) Supracoracoid muscle (poultry)	**Inorganic EDC determination**: The same profile of inorganic assessments for clinical biochemistry differential diagnostics as detailed for the inorganic water samples was performed using ICP-MS: Submitted to the ISCW (ARC).

For histopathology of the sentinel specimens the processed sections were examined by a specialist pathologist using standard techniques. For tissue values the processed specimen samples were pooled as observations per site and compared with reference values for broiler and poultry tissue values for inorganic constituents. Although this may be expanded for further assessments of deficiencies, adequacies and excesses, the objective of this investigation was to focus on differences between sites that may be reflected by histopathological observations and supported by blood hormone values. Median values were used for comparisons as opposed to mean values due to the stochastic variability inherent in tissue element values.

For the porcine samples an array of biomedical and commercial reference point values were used to assess the analytical results. The tests employed for thyroxine were adapted during the course of the project to suit the analytical test kits available and were conducted at the Veterinary Hormone Laboratory of the Faculty of Veterinary Science at the University of Pretoria. The histopathology was conducted at the Department of Paraclinical Sciences of the Faculty of Veterinary Science at the University of Pretoria.

The use of animal models for assessing agricultural chemicals and EDCs is arguably a preferable route as many of the key effects attributed to the potential types of effects are reported in the scientific literature in animal models. The use of animal models as sentinels for monitoring within the context of public health is also generally accepted. Fundamentally, the primary benefit is from a reduction in variation which contributes to confidence in attributing potential chemical/constituent effects to the exposures described to these chemicals/constituents.

The use of broilers was chosen as in many cases these systems are breed-specific and due to the commercial application multiple sites across South Africa will employ standard management systems, from health care, biosecurity, nutrition to environmental variables. This allows for meaningful extrapolation across differing agricultural exposure sites. Critically, between production system sites and farms, water intake is often the only single variable, with all other inputs standardized. These system types are thus specific and provide detail that not only serves as an input for exposure information, but also includes ingestion data. Exposure assessment is thus an actual measurement based on a high number of replicates and repetitions which allow for correction of environmental variables and influences (e.g. seasonal effects) accurately measured and not merely an estimate thereof. The key benefit is that aspects of source, pathway and exposure, may be isolated and controlled and accurately sampled and assessed. This is seldom, if ever, the case in human exposure studies. Intensive systems are also characterized by high biosecurity measures and herd health disease controls allowing for quick comparisons between sample groups and sites in terms of normality, and as such allow for significant uncertainty reduction. The significant reduction in confounding factors and exposure certainty increases the confidence in linking observed the effects to exposure.

The Letsitele sampling area did not present with a commercial broiler production unit, which is often present in rural villages in the form of the DAFF-sponsored village agricultural production projects. Consequently, the variation reduction benefit seen with commercial systems is not a major benefit with specimens collected from rural villages. Advantages to the rural specimens are that they represent specimens with longer exposure times and thus cumulative long-term effects may be evident histopathologically and in tissue values obtained. In addition, they represent an exposure receptor type characteristic of the village environment. Lastly, the tissue values offer information regarding community ingestion exposure pathways, as the poultry typically form a frequent part of the household diet, and as noted earlier, the use of a porcine model accords with the benefits from a commercial piggery similar to a commercial broiler system and the use thereof for biomedical research in human health studies.

Table 8.2: An overview of samples collected for the animal health assessment

Overview

• **Water** Quality for Source, Storage and Point of Use:
  o Full inorganic chemistry
  o Microbiological indicator organisms
  o Oestrogenic and Androgenic bioassays
  o Variable Physico-chemistry (site dependent)
  o Organic constituents (pesticide screening and selected quantitative tests)
• **Tissue** Samples:
  o 10 broiler specimens per site (commercial 35 days of age, rural variable):
  ▪ Thymus (bilateral) - Histopathology
  ▪ Thyroid & parathyroid (bilateral) – Histopathology
  ▪ Bursa of Fabricius – Histopathology
  ▪ Gonads (Testes) – Histopathology
  ▪ Liver – inorganic trace ICP-MS
  ▪ Deep Pectoral – inorganic ICP-MS
  ▪ Venous blood (brachial vein) – whole blood for thyroxine (T4)
  o 8 Porcine specimens from one site
  ▪ Serum Thyroxine
  ▪ Right Kidney - inorganic ICP-MS
  ▪ Caudate lobe of Liver - inorganic ICP-MS
  ▪ Thyroid - inorganic ICP-MS
  o Evaluation of each specimen for gross macroscopic lesions
• **Sediment** samples for Oestrogenic, Androgenic and Anti-androgenic bioassays
• **Wastewater samples:**
  o Liquid and Solids Fractions (as per WRC Technical Guidelines stipulated for Section 21 (e) and (g) activities.
• **Air** samples for organic screening and dispersion modelling (refer to CHAPTER 4:)

8.2.3 Analytical Procedures

8.2.3.1 Macro and Trace element analysis via IC, ICP-OES and ICP-MS

The chemical analyses of the water samples collected were conducted by IC, ICP-OES and ICP-MS techniques by the Institute for Soil Climate and Water at the Agricultural Research Council, Pretoria, using full quantitative and semi-quantitative procedures (ISCW, 2001 - AGRILASA). The anions are determined via IC, the cations via ICP-OES and the trace elements via ICP-MS. A separate run is conducted for iron due to interference from the standards used in the ICP-MS. Further methodology may be found in Standard Methods for the Examination of Water and Wastewater (1985) 16th Edition, American Public Health Association (ISBN: 0-87553-131-8).

Additional description of semi-quantitative and full quantitative ICP-MS methodology is presented in a separate Water Research Commission Report: K8/999: “Sampling and Methods for the Analysis of Inorganic Endocrine Disrupting Chemicals”. April 2012.

8.2.3.2 Physico-chemical procedures

The physico-chemical parameters were conducted by the Water Chemistry Section at the South African Bureau of Standards in accordance with SANS 241:2011 (Edition 1) (Aquakem-colimetric – ammonia; SANS 5213: 2005 – Chemical Oxygen Demand; SANS 6049: 2004 – Suspended Solids; SANAS TOO8 – Surfactants).

8.2.4 General Assessment Approaches

Fitness for use for animal watering is assessed using a combination of developmental constituent ingestion rate risk assessment procedures developed for the Water Research Commission Final Reports 644/1/98, 644/2/98, 644/3/98 and 857/1/01, 857/2/01 (Casey *et al*., 1998 Volumes 1, 2 and 3; Casey and Meyer, 2000; Casey *et al*., 2000) on a generic guideline application level and relevant recent international literature. This application level is concentration-based, as opposed to ingestion based, and is therefore conservative in risk estimation. A more accurate risk assessment may be performed when intake of a constituent is known from feed, water and other sources, and combined with site-specific risk factors that may increase, or decrease, the concentration at which a constituent in the water source may have an adverse effect. Use of specific information in this manner forms part of an ingestion-based specific guideline application level and hence involves site investigations with various data capturing procedures.

It should be taken into consideration that even the WHO Drinking Water Guidelines also recommend site-specific investigation as the next step following the presentation of water quality constituent values at concentrations exceeding the guidelines. This is in recognition of the limited application of table-based guidelines outside of relevant site-specific risk factors and accords with the general approach to hazardous substance exposure assessments in a sources, pathways and receptor description. Two aspects must be noted. Firstly, elevated water intakes due to osmo-sodium responses, high ambient temperatures, and palatability responses, significantly increase the risk for toxicity at any given guideline value (most are based on an average temp of 16 ºC). Secondly, combinations of hazardous constituents in the same water source can be synergistic, or antagonistic, and as such, end effects may mitigate, or exacerbate, toxicity.

Few water quality guidelines comment on recommended levels of water and diet/feed/forage levels simultaneously. Dual incorporation of these factors is generally impractical, for example, the high variability between breeds and species negate incorporation thereof into broad-based guidelines. It is also typical to encounter mixed animal breeds in the rural setting which is in stark contrast to then high selection pressure in single breed orientated intensive commercial production systems.

A generic level risk assessment is recommended as the first step in determining baseline exposures required for the identification of constituents in the geochemical environment that may contribute to adverse effects on health, productivity, and product quality. Any potential hazards identified would then require further

investigation regarding the water, user, environment and nutrition. It is crucial to note that for rural communal livestock production systems and domestic use that the lack of a dietary dilution factor if frequently relevant and an additional risk factor. The use of a water source with potentially hazardous chemical constituents may thus present with a higher risk as the same source may be simultaneously utilized for animal production, community or house-hold edible crop production and domestic purposes. Subsequent bioaccumulation factors and exposure via multiple pathways may thus result in a more sensitive user group category. As an example, lead is a recognized endocrine disrupting constituent and may be present in the drinking water for cattle at a level that does not present with any clinical symptoms in bovines, yet the concentration and form thereof in cow milk may pose significant risk to human consumption in the rural communal context.

In the rural setting dual exposure of agricultural chemicals in the drinking water may be relevant to both animals and humans. The general assessment of fitness for use for domestic purposes was conducted with the use of the following sets of guidelines in addition to relevant current international literature:

- DWAF (1996). Department of Water Affairs and Forestry (1996). South African Water Quality Guidelines (second edition) Volume 1. Domestic Use.
- SANS 241:2011 Edition 1. South African National Standard. Drinking Water. Part 1: Microbiological, physical, aesthetic and chemical determinands.
- QDWS (1998). Quality of Domestic Water Supplies. Volume 1. Assessment Guide. WRC TT 101/98. Published by Water Research Commission, The Department of Water Affairs and Forestry and The Department of Health.

It should be noted that the QDWS (1998) is based on the DWAF (1996) guidelines and that the SANS 241:2011 Standards are applicable to the point of delivery and are therefore adhered to by water services institutions and water services intermediaries. Although thus not strictly appropriate for the conditions of use in the rural community for the sampling phases conducted for this project the constituents and related guidelines do provide a current version of fitness for use assessments and are thus deemed sufficiently appropriate for inclusion.

As previously noted, the fundamental approach to assessment follows a source, pathway and receptor approach. The bulk of the data gathered during this project may be considered to be source analysis. This may also be thought of as Hazard Identification. It remains a central departure point in hazard and risk assessment to determine exposure based on knowledge of the source of agricultural chemicals in question. For a hazard to be used in the context of a descriptive term that characterizes the intrinsic capability thereof to cause harm the agricultural chemical must first of all be present.

Source description needs to be performed sufficiently to be able to contribute to exposure-response relationships in both field and epidemiology investigations that can yield credible risk assessments. Exposure data needs to consider both historical and geographical trends over time, and current exposure values. This is particularly challenging as the fate and transport of existing chemical through various source

media (water, sediment, and biota) are complex. In many cases the information used for reference purposes for source behaviour is obtained from chemical spills which are hard to extrapolate to low-concentration long-term source concerns.

To determine the possible routes of exposure the behaviour of the agricultural chemical being evaluated in terms of potential transport from the source to the receptor in question must be also evaluated. This pathway analysis may be thought of as exposure assessment and also takes into account the actual level of intake involved. It is also generally recognized that both the magnitude of exposure and relative potencies of multiple chemicals need to be considered. This implies that the context of exposure cannot be only considered for one aspect of exposure, but needs to be seen in context of other sources as well. For human health this implies that exposure to agricultural chemicals in the environment need to be seen in conjunction with exposure to EDCs in the home and workplace. For some agricultural workplace settings these exposure points may be the same, whilst for others the place of residence may differ significantly from the workplace.

This is becoming increasingly complicated by the general recognition that all routes of exposure need to be considered (dermal, inhalation, and ingestion) for multiple chemicals as the interactions may affect receptor events. Accordingly, the use of sentinel sampling was adopted to provide meaningful insight for the final outcome following exposure, as the screening and bioassay tools employed do not by themselves indicate true risk of an adverse effect following exposure to a sample providing a positive result for either presence of a pesticide or a specific cellular EDC response. The assessment of the final physiological significance was thus performed by observing key selected events in sentinel species.

It follows that exposure assessment should also place emphasis on vulnerable groups that represent critical development stages (gestation, lactation, adolescence, and senescence). This is in part recognition that the chemicals involved may affect cell proliferation, differentiation and organ development, hence exposure during sensitive periods provide the most significant potential for adverse effects. The type of receptor chosen determines receptor analysis and may be seen as the next logical step to determine the possibility for adverse effects following exposure. It should be noted that it was not possible to comprehensively investigate these receptor events for this project, but rather key research gaps and the justification thereof is presented.

A variety of local and international guidelines and limits for wastewater were used to determine classification of the applicable fractions. For water samples, concentrations recorded which exceed the Department of Water Affairs and Forestry (DWAF, 1996) guideline for the category C, or the internationally recommended upper guideline limit, or the SANS 241:2011 (Part 1) limits, are reported as exceeding the guidelines.

The following acronyms apply:

Concentrations recorded which exceed the Department of Water Affairs and Forestry (DWAF, 1996) guideline for the category C, or the internationally recommended

upper guideline limit, are reported as potentially hazardous chemical constituents (PHCC). Constituents that recorded values within 10% of a guideline limit or may within normal stochastic variation for the constituent approach within 10% of a guideline limit are reported as constituents of concern (COC).

Definitions of these terms appear below.

WQC:	Water quality constituent, e.g. Arsenic.
PHCC:	Indicates that exposure to the WQC in question is likely to result in adverse effects.
COC:	Indicates that the WQC in question could conceivably become a PHCC due to concentration variations, such as seasonal fluctuation in the water source or evaporative effects, and should therefore be monitored.

Additional relevant assessment acronyms include:

TWQR	target water quality range (DWAF, 1996)
AV	antagonistic variable (Underwood and Suttle, 1999)
Tox	Toxicity
Pal	Palatability

8.2.5 Method Approach Summary

As detailed in the National Water Act (No. 36 of 1998) the water resource classification system balances competing priorities for access to water quality and quantity. As causal links between exposure to agricultural chemicals in water and health related endpoints become increasingly established it follows that formulating resource quality objectives to define the desired management class also requires guidance in terms of the agricultural chemicals present in water. Although various aspects of this are dealt with in other Chapters the inputs to the processes defined therein rely on the ability to detect, screen, perform appropriate analytical techniques and assess the potential effects following exposure to agricultural chemicals. The methodology thus selected for the animal health assessment attempted to provide guidance on a process that could be applied to further studies on agricultural chemicals. Additional background information may be obtained from Meyer and Casey (2012) and the WRC reports noted in this Chapter. This is presented in more detail under the Recommendations section of this Chapter.

8.3 KEY RESULTS

The full results were presented in the various deliverables submitted for the project with only the key summary values presented here. The reader is referred to the

deliverables for sample specific fitness for use assessments and discussions thereon. As the final sampling phase addressed subterranean sources in the relevant sites the fitness for use assessments are presented here. Since the key PHCC noted and investigated was bromide, mean and standard deviation summary values are presented for this constituent only.

8.3.1 Water Quality

Refer to CHAPTER 2: for details of boreholes sampled in each of the study areas.

8.3.1.1 Inorganic Chemistry Results – Letsitele

Table 8.3: Macro-constituent results for samples collected from boreholes in the Letsitele catchment (mg/L)

Constituent	PS BH1	PS BH2	MA BH1
Fluoride	0.55	0.11	0.2
Nitrite	0.88	0	0.07
Nitrate	1.87	6	48
Chloride	34	31	27
Sulphate	5.98	2.9	0.8
Phosphate	0	0.45	0.78
Bicarbonate	246	234	34
Sodium	31	7	20
Potassium	2	0.6	1.61
Calcium	65	12	10
Magnesium	11	50	11
pH	8.25	8.27	6.95
TDS	275	228	139
TH	208	238	76
SAR	0.95	0.21	1.03

Table 8.4: Trace-constituent results for samples collected from boreholes in the Letsitele catchment (µg/L)

Constituent	PS BH1	PS BH2	MA BH1
Arsenic	0	0	0
Beryllium	0	0	0
Bismuth	0	0	0
Barium	7	8	26
Bromide	95	68	118
Cadmium	0	0	0
Copper	1	6	5
Cobalt	0	0	0
Chromium	1	1	6
Caesium	0.5	0.1	0
Iodine	100	1	9
Lead	0	0	0
Lanthanum	0	0	0
Lithium	10	0.4	8
Mercury	0	0	0
Manganese	126	1	5
Molybdenum	0	0	0
Nickel	0.9	4	2
Selenium	0.4	0.3	0.8
Strontium	785	43	71
Tellurium	0	0	0
Thallium	0	0	0
Titanium	2	5	2
Antimony	0	0	0
Tin	0.4	1.9	0.6
Tungsten	0	0	0
Uranium	0	0	0
Vanadium	0.3	45	0.6
Zinc	0	0.4	5
Iron	100	100	160

Table 8.5: Alkalinity and buffering results for samples collected from boreholes in the Letsitele catchment.

Parameter	PS BH1	PS BH2	MA BH1
Sodium Carbonate	0	0	0
Sodium Bicarbonate	53	6	0
Alkalinity	202	192	28
Temporary Hardness	202	192	28
Permanent Hardness	7.8	46	48

Table 8.6: Physicochemical results for samples collected from boreholes in the Letsitele catchment.

Site	Chemical Oxygen Demand (as O_2 in mg/L)	Surfactants (mg/L)	Free and Saline Ammonia (as N mg/L)	Suspended solids at 105 degrees C (mg/L)
PS BH1	<10	<0.1	<0.3	<5
PS BH1	<10	<0.1	<0.3	<5
MA BH1	<10	<0.1	<0.3	<5
Relevant Guideline	TWQR = 6 - 9	TWQR = <0.1	TWQR = <0.3	TWQR = <3

8.3.1.2 Inorganic Chemistry Fitness for Use (Livestock) – Letsitele

Table 8.7: Inorganic chemistry fitness for use results for PS BH1

PHCCs (mg/L)				COCs (mg/L)			
WQC	**Observed Value**	**Relevant Guideline**	**Norms affected**	**WQC**	**Observed Value**	**Relevant Guideline**	**Norms affected**
Br	0.095	TWQR 0- 0.01	Health – Tox	Sr	0.785	<0.1	AV
Mn	0.126	TWQR 0-0.1	Health – Pal				
I	0.100	TWQR 0-0.1	Health – Tox				
Fe	0.100	TWQR 0-0.02	Health – Pal				
TH ($CaCO_3$)	208	TWQR 50-150	Water System - Scaling				

Table 8.8: Inorganic chemistry fitness for use results for PS BH2

PHCCs (mg/L)				COCs (mg/L)			
WQC	**Observed Value**	**Relevant Guideline**	**Norms affected**	**WQC**	**Observed Value**	**Relevant Guideline**	**Norms affected**
Br	0.068	TWQR 0-0.01	Health – Tox				
Fe	0.100	TWQR 0-0.02	Health – Pal				
TH ($CaCO_3$)	238	TWQR 50-150	Water System - Scaling				

Table 8.9: Inorganic chemistry fitness for livestock use results for MA BH1

PHCCs (mg/L)				COCs (mg/L)			
WQC	**Observed Value**	**Relevant Guideline**	**Norms affected**	**WQC**	**Observed Value**	**Relevant Guideline**	**Norms affected**
Br	0.118	TWQR 0-0.01	Health – Tox				
NO_3	48	TWQR 0-26	Health – Tox				
Fe	0.160	TWQR 0-0.02	Health – Pal				

8.3.1.3 Inorganic Chemistry Fitness for Use (Domestic Use) – Letsitele

Table 8.10: Inorganic chemistry fitness for domestic use

Sample	PHCC & COC	Comments
PS BH1	Br	Exceeds MCL (disinfection by-product hazard).
	TH	Exceeds TWQR, Scaling.
	Fe	Exceeds TWQR, Marginal aesthetic effects.
	Mn	Exceeds TWQR, marginal risk.
PS BH2	Br	Exceeds MCL (disinfection by-product hazard).
	TH	Exceeds TWQR, Scaling
	Fe	Exceeds TWQR, Marginal aesthetic effects.
MA BH1	Br	Exceeds MCL (disinfection by-product hazard)
	TH	Exceeds TWQR, Scaling
	NO_3	Exceeds TWQR, significant health effects.
	Fe	Exceeds TWQR, Marginal aesthetic effects.

8.3.1.4 Inorganic Chemistry Results – Lomati

Table 8.11: Macro-constituent results for samples collected from boreholes in the Lomati catchment (mg/L)

Constituent	GW	RHL	KC
Fluoride	0.86	1.66	1.03
Nitrite	0	6	6.57
Nitrate	26	20	19
Chloride	1492	617	151
Sulphate	131	44	32
Phosphate	6	4.9	10.2
Bicarbonate	139	319	389
Sodium	474	210	72
Potassium	1.3	0.7	0.2
Calcium	411	162	94
Magnesium	45	39	96
pH	7.72	7.81	8.21
TDS	2660	1261	639
TH	1223	571	468
SAR	5.9	3.83	1.45

Table 8.12: Trace-constituent results for samples collected from boreholes in the Lomati catchment (µg/L)

Constituent	GW	RHL	KC
Arsenic	3.7	0.1	0
Beryllium	0	0	0
Bismuth	0	0	0
Barium	0	6.8	0
Bromide	2640	1116	721
Cadmium	0	0	0
Copper	1	2	23
Cobalt	0	0	0
Chromium	1	2	2
Caesium	0	0	0
Iodine	1110	243	36
Lead	0	0	1.7
Lanthanum	0	0	0
Lithium	51	137	14
Mercury	6.6	1.6	0
Manganese	7	183	0.5
Molybdenum	0	0	1
Nickel	8	4	2
Selenium	17	7	4
Strontium	415	331	269
Tellurium	0	0	0
Thallium	0	0	0
Titanium	4	3	3
Antimony	0	0	0
Tin	0	0	3
Tungsten	0	0	0
Uranium	1	1	0
Vanadium	26	15	37
Zinc	2	14	33
Iron	70	70	60

Table 8.13: Alkalinity and buffering results for samples collected from boreholes in the Lomati catchment.

Parameter	GW	RHL	KC
Sodium Carbonate	0	0	0
Sodium Bicarbonate	0	0	0
Alkalinity	114	261	319
Temporary Hardness	114	261	319
Permanent Hardness	1109	310	149

8.3.1.5 Inorganic Chemistry Fitness for Use (Livestock) – Lomati

Table 8.14: Inorganic chemistry fitness for use results for GW

PHCCs (mg/L)				COCs (mg/L)			
WQC	**Observed Value**	**Relevant Guideline**	**Norms affected**	**WQC**	**Observed Value**	**Relevant Guideline**	**Norms affected**
Br	2.640	TWQR 0-0.01	Health – Tox	Sr	0.415	AV <0.1	Health
NO_3	26	TWQR 0-26	Health – Tox	As	0.0037	TWQR 0-0.01	Health – Tox
Na	474	TWQR 0-50	Health – Tox & Pal	F	0.86	TWQR 0-1	Health – Tox
Cl	1492	TWQR 0-14 (Na> 50 mg/L)	Health – Tox				
Mg	45	TWQR 0-30	Health – Tox & Pal				
Se	0.017	TWQR 0-0.01	Health – Tox				
Hg	0.0066	TWQR 0-0.002	Health – Tox				
I	1.110	TWQR 0-0.1	Health – Tox				
Fe	0.070	TWQR 0-0.02	Health – Pal				
Ca	411	TWQR 0-150	Health – Tox & Pal				
TDS	2660	TWQR 0-1000	Health – Pal				
TH ($CaCO_3$)	1223	TWQR 50-150	Water System - Severe Scaling	Nutrient Loading Impact: PO_4 = 6			

Table 8.15: Inorganic chemistry fitness for use results for RH

PHCCs (mg/L)				COCs (mg/L)			
WQC	**Observed Value**	**Relevant Guideline**	**Norms affected**	**WQC**	**Observed Value**	**Relevant Guideline**	**Norms affected**
Br	1.116	TWQR 0-0.01	Health – Tox	Sr	0.331	AV <0.1	Health
Na	210	TWQR 0-50	Health – Tox & Pal	Se	0.007	TWQR 0-0.01	Health – Tox
Cl	617	TWQR 0-14 (Na> 50 mg/L)	Health – Tox	NO_3	20	TWQR 0-26	Health – Tox
Mg	39	TWQR 0-30	Health – Tox & Pal	NO_2	6	TWQR 0-10	Health – Tox
Mn	0.183	TWQR 0-0.1	Health – Pal	Hg	0.0016	TWQR 0-0.002	Health – Tox
F	1.66	TWQR 0-1	Health – Tox				
I	0.243	TWQR 0-0.1	Health – Tox				
Fe	0.070	TWQR 0-0.02	Health – Pal				
Ca	162	TWQR 0-150	Health – Tox & Pal				
TDS	1261	TWQR 0-1000	Health – Pal				

TH ($CaCO_3$)	571	TWQR 50-150	Water System - Severe Scaling	Nutrient Loading Impact: PO_4 = 4.9

Table 8.16: Inorganic chemistry fitness for use results for KC

PHCCs (mg/L)				COCs (mg/L)			
WQC	**Observed Value**	**Relevant Guideline**	**Norms affected**	**WQC**	**Observed Value**	**Relevant Guideline**	**Norms affected**
Br	0.721	TWQR 0-0.01	Health – Tox	Sr	0.269	AV <0.1	Health
Na	72	TWQR 0-50	Health – Tox & Pal	Se	0.004	TWQR 0-0.01	Health – Tox
Cl	151	TWQR 0-14 (Na> 50 mg/L)	Health – Tox	NO_3	19	TWQR 0-26	Health – Tox
Fe	0.060	TWQR 0-0.02	Health – Pal	NO_2	6.5	TWQR 0-10	Health – Tox
Mg	96	TWQR 0-30	Health – Tox & Pal	Pb	0.002	TWQR 0-0.01	Health – Tox
Mn	0	TWQR 0-0.1	Health – Pal				
F	1.03	TWQR 0-1	Health – Tox				
TH ($CaCO_3$)	468	TWQR 50-150	Water System - Severe Scaling	Nutrient Loading Impact: PO_4 = 10.62			

8.3.1.6 Inorganic Chemistry Fitness for Use (Domestic Use) – Lomati

Table 8.17: Inorganic chemistry fitness for domestic use

Site	PHCC & COC	Comments
GW	Br	Exceeds MCL (disinfection by-product hazard)
	NO_3	Exceeds TWQR, marginal risk
	Fe	Exceeds TWQR, Marginal aesthetic effects
	Hg	Exceeds TWQR, Potential adverse effects in all users.
	Se	Exceeds TWQR, Potential adverse effects in all users.
	Ca	Exceeds TWQR, Chronic health effects in sensitive users.
	PO_4	Exceeds naturally occurring values in water by a significant margin. Indicative of potential contaminant pollution pathway.
	TDS	Exceeds TWQR, Possible health risks to all individuals
	Cl	Exceeds TWQR, Dehydration in infants, nausea and vomiting.
	TH	Exceeds TWQR, Chronic effects in sensitive user groups.
RH	Br	Exceeds MCL (disinfection by-product hazard)
	NO_3	Exceeds TWQR, marginal risk
	Fe	Exceeds TWQR, Marginal aesthetic effects
	F	Exceeds TWQR, Adverse health effects in all individuals.
	Hg	Exceeds TWQR, Potential adverse effects in all users.
	Mn	Exceeds TWQR, Marginal aesthetic effects.
	Ca	Exceeds TWQR, Chronic health effects in sensitive users.
	PO_4	Exceeds naturally occurring values in water by a significant

Site	PHCC & COC	Comments
		margin. Indicative of potential contaminant pollution pathway.
	TDS	Exceeds TWQR, Possible health risks in sensitive users.
	Cl	Exceeds TWQR, Possible long-term health effects.
	TH	Exceeds TWQR, Possible chronic effects in sensitive users.
	Br	Exceeds MCL (disinfection by-product hazard)
	NO_3	Exceeds TWQR, marginal risk
	NO_2	Constituent of Concern.
	Fe	Exceeds TWQR, Marginal aesthetic effects
	F	Exceeds TWQR, Adverse health effects in all individuals.
KC	Mg	Exceeds TWQR, Marginal aesthetic effects.
	PO_4	Exceeds naturally occurring values in water by a significant margin. Indicative of potential contaminant pollution pathway.
	TDS	Exceeds TWQR, Possible health risks in sensitive users.
	Cl	Exceeds TWQR, Possible long-term health effects.
	TH	Exceeds TWQR, Possible chronic effects in sensitive users.

8.3.1.7 Inorganic Chemistry Results – Vals and Renoster

Table 8.18: Macro-constituent results for samples collected from boreholes in the Vals and Renoster catchments (mg/L)

	VSK	KSD	KOP
Fluoride	0.3	0.38	0.36
Nitrite	2.8	0.5	1
Nitrate	46	0.6	42
Chloride	70	11.9	97
Sulphate	40	53	33
Phosphate	5.88	0.4	0.53
Carbonate	0	0	0
Bicarbonate	245	241	471
Sodium	35	21	126
Potassium	4.8	0.8	14.2
Calcium	81	54	81
Magnesium	31	29	34
pH	8.14	8.25	8.19
TDS	442	294	667
TH	332	259	349
SAR	0.85	0.59	2.95

Table 8.19: Trace-constituent results for samples collected from boreholes in the Vals and Rensoter catchments (µg/L)

Constituent	VSK	KSD	KOP
Arsenic	0	0	0
Beryllium	0	0	0
Bismuth	0	0	0
Barium	0	10	22
Bromide	48	29	460
Cadmium	0	0	0
Copper	0	0	2
Cobalt	0	0	0
Chromium	1	1	2
Caesium	0.5	0	0
Iodine	14	30	29
Lead	0	0	0
Lanthanum	0	0	0
Lithium	5	12	34
Mercury	0	0	0
Manganese	0	0	0
Molybdenum	0	0	0
Nickel	1	1	1.7
Selenium	0	0	4
Strontium	280	212	633
Tellurium	0	0	0
Thallium	0	0	0
Titanium	1	2	1
Antimony	0	0	0
Tin	0	1	0
Tungsten	1	0	0
Uranium	2	0.9	1
Vanadium	5	9	5
Zinc	0	2	307
Iron	60	70	70

Table 8.20: Alkalinity and buffering results for samples collected from boreholes in the Vals and Rensoter catchments.

Parameter	VSK	KSD	KOP
Sodium Carbonate	0	0	0
Sodium Bicarbonate	0	0	62
Alkalinity	201	198	386
Temporary Hardness	201	198	349
Permanent Hardness	131	61	0

Table 8.21: Physicochemical results for samples collected from boreholes in the Vals and Rensoter catchments.

Site	Chemical Oxygen Demand (as O_2 in mg/L)	Surfactants (mg/L)	Free and Saline Ammonia (as N mg/L)	Suspended solids at 105 degrees C (mg/L)
VJK	<10	<0.1	<0.3	<5
KSD	<10	<0.1	<0.3	<5
KOP	<10	<0.1	<0.3	<5
Relevant Guideline	TWQR = 6 - 9	TWQR = <0.1	TWQR = <0.3	TWQR = <3

8.3.1.8 Inorganic Chemistry Fitness for Use (Livestock) – Lomati

Table 8.22: Inorganic chemistry fitness for use results for VJK

PHCCs (mg/L)				COCs (mg/L)			
WQC	**Observed Value**	**Relevant Guideline**	**Norms affected**	**WQC**	**Observed Value**	**Relevant Guideline**	**Norms affected**
Br	0.048	TWQR 0-0.01	Health – Tox	Sr	0.280	AV <0.1	Health
NO_3	46	TWQR 0-26	Health – Tox				
Fe	0.060	TWQR 0-0.02	Health – Pal				
Mg	31	TWQR 0-30	Health – Tox & Pal				
TH ($CaCO_3$)	332	TWQR 50-150	Water System - Scaling	Nutrient Loading Impact: PO_4 = 5.88			

Table 8.23: Inorganic chemistry fitness for use results for KSD

PHCCs (mg/L)				COCs (mg/L)			
WQC	**Observed Value**	**Relevant Guideline**	**Norms affected**	**WQC**	**Observed Value**	**Relevant Guideline**	**Norms affected**
Br	0.029	TWQR 0-0.01	Health – Tox	Sr	0.212	AV <0.1	Health
Fe	0.070	TWQR 0-0.02	Health – Pal	Mg	29	TWQR 0-30	Health – Tox & Pal
TH ($CaCO_3$)	259	TWQR 50-150	Water System - Scaling				

Table 8.24: Inorganic chemistry fitness for use results for KOP

PHCCs (mg/L)				COCs (mg/L)			
WQC	**Observed Value**	**Relevant Guideline**	**Norms affected**	**WQC**	**Observed Value**	**Relevant Guideline**	**Norms affected**
Br	0.460	TWQR 0-0.01	Health – Tox	Sr	0.633	AV <0.1	Health
NO_3^-	42	TWQR 0-26	Health – Tox	Zn	0.307	AV <0.1	Health
Na	126	TWQR 0-50	Health – Tox & Pal	Se	0.004	TWQR 0-0.01	Health – Tox
Cl	97	TWQR 0-14 (Na> 50 mg/L)	Health – Tox				
Mg	34	TWQR 0-30	Health – Tox & Pal				
Fe	0.070	TWQR 0-0.02	Health – Pal				
TH ($CaCO_3$)	349	TWQR 50-150	Water System - Scaling	Nutrient Loading Impact: PO_4^{3-} = 0.53 K^+ = 14.2			

8.3.1.9 Inorganic Chemistry Fitness for Use (Domestic Use) – Lomati

Table 8.25: Inorganic chemistry fitness for domestic use

Sample	PHCC & COC	Comments
VJK	Br	Exceeds MCL (disinfection by-product hazard)
	NO_3^-	Significant Health Risk (drinking and food preparation)
	Fe	Exceeds TWQR, Marginal aesthetic effects
	TH	Scaling
	PO_4^{3-}	Exceeds naturally occurring values in water. Indicative of potential contaminant pollution pathway.
KSD	Br	Exceeds MCL (disinfection by-product hazard)
	Fe	Exceeds TWQR, Marginal aesthetic effects
	TH	Scaling
KOP	Br	Exceeds MCL (disinfection by-product hazard)
	NO_3^-	Significant Health Risk (drinking and food preparation)
	Fe	Exceeds TWQR, Marginal aesthetic effects
	TH	Scaling
	PO_4^{3-} + K^+	Exceeds naturally occurring values in water. Indicative of potential contaminant pollution pathway.

8.3.1.10 Additional Agricultural Impact Related Constituents

As a general summary for the sampling observations it may be considered that the following constituents assist with determining the likelihood of the agricultural water use activity impacting the available water resources.

It should be noted that several other macro and trace elements may be increased, depending on the type of agricultural chemicals used, but the ones presented here

appear to be the key inorganic and physic-chemical markers that if triggered would justify further sampling (e.g. pesticides and pharmaceuticals if relevant). The use of microbiological indicator organisms is also generally included where confined animal feeding operations are applicable (Section 21 a, e and g Activities of the National Water Act).

Table 8.26: NPS impact constituents (mg/L)

	NO_2	NO_3	PO_4	K^+	NH_3	COD	Surfactants
PSBH1	0.88	1.87	0	2	<0.3	<10	<0.1
PSBH2	0	6	0.45	0.6	<0.3	<10	<0.1
MABH1	0.07	48	0.78	1.61	<0.3	<10	<0.1
KGWBH1	0	26	6	1.3	<0.3	13	<0.1
KRHL	6	20	4.9	0.7	<0.09	<10	<0.1
KRLBH	6.57	19	10.2	0.2	<0.09	<10	<0.1
VSK	2.8	46	5.88	4.8	<0.09	<10	<0.1
KSD	0.38	0.6	0.4	0.8	<0.3	<10	<0.1
KOP	0.36	42	0.53	14.2	<0.3	<10	<0.1

Table 8.27: Trace contaminant constituents (µg/L)

	As	Cd	Cu	Pb	Mn	Ni	Se	Zn
PSBH1	0	0	1	0	126	0.9	0.4	0
PSBH2	0	0	6	0	1	4	0.3	0.4
MABH1	0	0	5	0	5	2	0.8	5
KGWBH1	3.7	0	1	0	7	8	17	2
KRHL	0.1	0	2	0	183	4	7	14
KRLBH	0	0	23	1.7	0.5	2	4	33
VSK	0	0	0	0	0	1	0	0
KSD	0	0	0	0	0	1	0	2
KOP	0	0	2	0	0	1.7	4	307

8.3.2 Surface Water Samples

8.3.2.1 Letsitele sampling phases

Table 8.28: Summary of inorganic chemistry results collected in the Letsitele catchment

Key Marker Constituents	Range for Samples (mg/L) [median and SD]				
	July 2011	**November 2011**	**June 2012**	**December 2012**	**April 2013**
NO_2^-	0	0	0-1.42	0	0-0.4
NO_3^-	0.9-62	0-51	0.24-0.5	0.03-0.63	0.1-0.5
$P0_4^{3-}$	0-4.1	0-0.3	0-0.16	0-0.04	0.23-0.4
K^+	0.3-1.9	0.2-2.1	0.4-1.5	0.8-2	1.6-2.9
COD	<10-65	<10-400	<10	<10	<10-311
NH_3	<0.3	<0.3	<0.3	<0.3	<0.3
Surfactants	<10	<10	<0.1-1.2	<0.1	<0.1
As	0	0	0	0-0.001	0-0..006
Br	[0.0375 ±0.0362]	[0 ±0.0197]	[0.057 ±0.048]	[0.016 ±0.012]	[0.027 ±0.0.0121]
Cd	0	0	0	0	0
Cu	0-0.037	0	0-0.072	0-0.012	0-0.016
Cs	0	0	0.002-0.153	0	0
Pb	0-0.001	0	0-0.0028	0-0.0007	0-0.001
Mn	0-1.350	0.001-0.085	0.006-0.152	0.002-0.169	0.008-0.107
Ni	0-0.019	0	0-0.397	0.001-0.003	0
Se	0-0.002	0-0.001	0	0-0.002	0-0.001
Zn	0.002-4.384	0-0.146	0-0.359	0-0.149	0-0.327

8.3.2.2 Lomati sampling phases

Table 8.29: Summary of inorganic chemistry results collected in the Lomati River catchment

Key Marker Constituents	Range for Samples (mg/L) [median and SD]			
	June 2012	**September 2012**	**December 2012**	**April 2013**
NO_2^-	0-1.04	0-0.03	0	0-0.25
NO_3^-	0.33-20.47	0.49-9.4	0.02-6.2	0.01-1
$P0_4^{3-}$	0	0	12-23.77	0-2.4
K^+	0-3.6	0.15-3.6	2-3.3	1.7-3.3
COD	12-68	<10-28	<10-28	<10-207
NH_3	<0.3	<0.3	<0.3	<0.3
Surfactants	<0.1	<0.1	<0.1	<0.1
As	0.001-0.0019	0-0.001	0.001-0.004	0-0.001
Br	[0.219 ±0.093]	[0.076 ±0.134]	[0.046 ±0.049]	[0.058 ±0.077]
Cd	0	0	0	0
Cu	0-0.002	0-0.011	0-0.002	0-0.009
Cs	0	0	0	0-0.001
Pb	0	0	0-0.001	0
Mn	0.025-0.238	0.017-0.103	0.039-0.169	0.007-0.086
Ni	0-0.005	0-0.002	0.005-0.013	0-0.002
Se	0	0	0-0.004	0-0.004
Zn	0.001-0.026	0.001-0.024	0.001-0.012	0-0.018

8.3.2.3 Vals River sampling phases

Table 8.30: Summary of inorganic chemistry results collected in the Vals River catchment

Key Marker Constituents	Range for Samples (mg/L) [median and SD]			
	July 2012	**October 2012**	**February 2013**	**June 2013**
NO_2^-	0-0.7	0.3-1.7	0-9.5	0.04-0.7
NO_3^-	2.1-94.7	0.9-82.1	0.1-34	0.1-5.4
$P0_4^{3-}$	0-9.63	0-6.02	- = 6.4	0.68-5.8
K^+	3.7-16.6	5.4-14.4	6.1-12.3	11-17.9
COD	<10-69	11-37	40 – 48	<10-381
NH_3	<0.3	<0.3	<0.3	<0.3
Surfactants	<0.1	<0.1-0.3	<0.1	<0.1
As	0.0015-0.004	0.003-0.008	0.003-0.01	0-0.002
Br	[0.332 ±0.117]	[0.293 ±0.058]	[0.116 ±0.025]	[0.032 ±0.0201]
Cd	0	0	0	0
Cu	0.001-0.003	0	0-0.001	0
Cs	0.0002-0.0005	0	0	0
Pb	0	0-0.001	0	0
Mn	0.017-1.008	0.167-0.669	0.001-0.382	0.018-0.337
Ni	0-0.018	0.005-0.006	0.010-0.020	0.001-0.006
Se	0-0.001	0.002-0.002	0.004-0.005	0-0.001
Zn	0.004-0.013	0.007-0.028	0-0.002	0.002-0.003

8.3.2.4 Renoster River sampling phases

Table 8.31: Summary of inorganic chemistry results collected in the Renoster River catchment

Key Marker Constituents	Range for Samples (mg/L) [median and SD]			
	July 2012	**October 2012**	**February 2013**	**June 2013**
NO_2^-	0	0-0.19	0-1	0
NO_3^-	0.7-9.6	0.5-2.8	0.7-1	0
$P0_4^{3-}$	0-0.27	0-0.124	0-1	0.045-1.48
K^+	3.6-6.1	3.5-7.9	6.2-9.2	1.9-5.8
COD	<10-27	20	14-22	36-72
NH_3	<0.3	<0.3	<0.3	<0.3-0.7
Surfactants	<0.1	0.2	<0.1	<0.1
As	0.001-0.0018	0.001-0.002	0.001-0.004	0
Br	[0.276 ±0.113]	[0.179 ±0.120]	[0.133 ±0.076]	[0042. ±0.052]
Cd	0	0	0	0
Cu	0-0.002	0-0.001	0-0.003	0-0.002
Cs	0.0009-0.0013	0	0	0
Pb	0	0-0.001	0-0.0008	0-0.0006
Mn	0.057-0.129	0.102-0.203	0.001-0.137	0.016-0.116
Ni	0-0.023	0 .003-0.007	0.010-0.012	0.003-0.010
Se	0	0-0.003	0-0.006	0-0.003
Zn	0-0.047	0.02-0.003	0-0.009	0.001-0.003

8.3.3 Wastewater Samples

Samples were obtained from three commercial confined animal feeding operations in order to assess the source, pathway and receptors relevant to the suspected PHCCs. Whilst a more detailed study was conducted, only the key results are presented here. It was noted that the use of anaerobic digestion and a separator stage that produced a solids fraction (subsequently composted) and a liquid fraction significantly improved the quality of that disposed to agricultural land in accordance with Section 21 (e) activities of the NWA.

Table 8.32: Liquid fraction results, post separation and anaerobic digestion.

Metals	Pollutant Class A (ppm)	Liquid Fraction Site 1 (ppm)	Liquid Fraction Site 2 (ppm)	Liquid Fraction Site 3 (ppm)
Arsenic	<40	0.019	0.013	0.025
Cadmium	<40	0	0	0.0019
Chromium	<1200	0.181	0.112	0.394
Copper	<1500	0.026	0.021	8.615
Lead	<300	0	0	0.012
Mercury	<15	0	0	0
Nickel	<420	0.254	0.171	0.446
Zinc	<2800	2.587	3.840	10.390

Microbiological Quality (Faecal Coliform c/100 mL):

- Raw – Separator**:** 67 500 – 4 360
  - **Benefit: 93.5% Reduction**

- Physico-chemical properties (mg/L):
  - Raw – Separator:
    - Suspended Solids: 24 000 – 15 700
      - **Benefit: 65% reduction**
    - Ammonia: 1 696 – 4 812
      - **Benefit: 183% increase**

The inorganic results noted indicate a benefit associated with the mineralization achieved with better aeration efficacy on the solids removal by the separator process.

- **Sludge Classification: Pollutant Class: A**:
  - Thus:
    - Agricultural Use is an acceptable option.
    - No additional restrictions or requirement apply.
    - Could be used as a saleable product.

8.4 TISSUE INVESTIGATIONS

8.4.1 Poultry Sentinel Assessment

Since the use of the Veterinary Diagnostic Laboratory for WRC Project K5/1915 (Meyer *et al*., 2014) for the assessment of plasma TT4 values, the kit used has changed. No reasons other than sample volume handling considerations were given for this, and a canine T4 kit was used instead. According to experts consulted from the USA via the consultant veterinary poultry pathologist the T4 structure is sufficiently similar to permit the kit to be used for poultry. In order to make the sample collection and analysis more robust it was decided to change from plasma to serum values with a horizontal tube storage leading to increased serum sample volume – the collections as conducted in the field led to some samples containing insufficient volume for the new kit.

Table 8.33: Comparisons between observed data and reference values for plasma Thyroxine (total T4).

Letsitele and Komatipoort Samples Plasma Thyroxine values (Total T_4) in nmol/L				
Observations*		**Reference: Ross^**	**Reference: Non Commercial^**	**Reference: Range for 6 commercial broiler breeds^**
Letsitele (n = 8)	**Komatipoort (n = 10)**			
2.70 ± 2.140	1.758 ± 2.278	10.89 ± 0.849	8.906 ± 1.081	9.715 ± 0.514 10.707 ± 0.489
Plasma Thyroxine values (Total T_4) in nmol/L at 1 day				
Reference: Ross^		**Reference: Non Commercial^**		**Reference: Range for 6 commercial broiler breeds^**
5.933 ± 0.785		5.997 ± 0.123		4.337 ± 0.694 6.151 ± 0.939

^ Adapted from Gonzales *et al*., (1999) n = 12 per breed. [71]

*No statistical procedure conducted due to sample integrity losses described in text.

Table 8.34: Histopathological observations in poultry specimens collected from the Letsitele and Komatipoort study areas.

Letsitele Site	Komatipoort Site
L1 Thymus – NA Thyroid – follicular structures normal, **colloid vacuolated**, pink in colour Bursa – Grade 2 to 4 **L2&3** Thymus – marked atrophy, loss of cortico medullary distinction Thyroid – scattered enlarged follicles filled with pink slightly vacuolated colloid **L4** Thyroid – follicular structures normal, colloid vacuolated, pink in colour	**Pooled sections (n = 10)** Thymus – NA Thyroid – varying sized follicles, deep pink colloid, and ***minimal vacuolation*** Bursas – Grade 4 Adrenal – NA Immature testes and ovaries – NA

Letsitele Site	Komatipoort Site
Thymus - NA Testes – NA **L5** Thymus – marked atrophy, loss of cortico medullary distinction Thyroid – scattered enlarged follicles filled with pink slightly vacuolated colloid	

Bursal grading:
0 = no damage
2 = moderate generalized lymphocyte depletion
3 = over 50% of follicles with severe lymphocyte depletion and
4 = outline of follicles, fibroplasia, cystic epithelium
5 = loss of all follicular architecture and fibroplasia

NA = No abnormalities

8.4.2 Porcine Sentinel Assessment

Following unresolved clinical issues in the breeding section of a commercial piggery in which suspected agricultural chemicals and EDCs had adversely affected subterranean drinking water, sampling was conducted to evaluate the health status linked to the suspected constituents. Bromide was identified as a possible causative constituent resulting in both thyroid dysfunction and induced trace element deficiencies. A follow up sampling phase was conducted after mitigation for the primary suspected causative constituent identified in the first phase, with a positive response to thyroid function being observed.

Details of samples obtained from the consultant veterinarian and processed prior to submission to the laboratory for inorganic chemical analysis are listed in Table 8.35. A total of six subterranean water samples and one surface water (receiving environment) sample were obtained for inorganic chemical analysis (Table 8.36).

Table 8.35: Porcine samples submitted for inorganic chemical analysis

Tissue Type	Initial Sampling (Diagnostic)	Post-mitigation Sampling
Kidney Samples:		
Right kidney	n = 6	n = 8
Liver Samples:		
Caudate Lobe	n = 5	n = 8
Thyroid gland:		
Entire Gland	n = 4	n = 6
Whole Blood:		
Lithium heparin	n = 6 x 2	n = 6*
Serum:		
Red (clot activator)	n = 6:	n = 6

8.4.2.1 Water Quality

Table 8.36: Summary of inorganic chemical analysis from six subterranean water samples and one surface water sample.

Key Marker Constituents	Range for Samples 1-6 (mg/L) [median and SD]	Sample 7 (mg/L)
NO_2	0.3-2.5	0
NO_3	6.8-91.6	2
$P0_4$	0	13.7
K^+	2.9-3.9	18.23
COD	-	140
NH_3	-	<0.09
Surfactants	-	0.6
Suspended Solids	-	84
As	0.001-0.002	0.0016
Br	0.113-0.367 [0.252 ±0.121]	0.201
Cd	0	0
Cu	0.0004-0.003	0
Cs	0	0
Pb	0-0.0017	0
Mn	0-0.503	0.676
Ni	0-0.021	0
Se	0.0002-0.0036	0.0018
Zn	0.001-0.268	0.001

Table 8.37: Microbiological quality results suggestive of potential agricultural activity impact.

Sample	Microbiological Indicator Organisms			
	Total Bacteria Count (Count/mL)	**Coliform Bacteria Count** (Count/100mL)	**Faecal Coliform Bacteria Count** (Count/100mL)	***E. coli*** (count/100mL)
6	**>30 000**	**548**	**12**	**19**
7	4 100	**>2 419**	4	41
Relevant Guidelines:	Risk = Operational Limit ≤1000	Risk = Operational Limit ≤ 10	Risk = Health Classification:	**Risk** = Acute Health
SANS 241: 2011 (Part 1)	(previous operational limit < 3000)	Risk = Health Classification: 0-10 Good	0 1 – Good	Limit 0 (not detected)
QDWS (1998)	Risk = Health – NA **Biofilm Hazard**	10-100 Marginal 100-1000 **Poor**	1-0 Marginal 10-100 **Poor** > 100 Completely Unacceptable	
		> 1000 **Completely Unacceptable**		

8.4.2.2 Tissue Results

Table 8.38: Porcine blood TT4 results pre and post mitigation

Phase 1 (pre-mitigation) TT4 (nmol/L)	**Phase 2 (9 months post mitigation) TT4 (nmol/L)**
49.8	40.2
28.4	30.5
28.8	45.5
27.0	21.9
44.5	40.9
30.4	35.3
Median & SD	Median & SD
29.6 ± 9.75	**37.75** ± 5.740

Table 8.39: Summary of inorganic constituents detected in porcine tissue samples.

Constituent	**Phase 1 (pre-mitigation)** **Summary Statistics** (mg/kg FW) **Median ± SD [range]**	**Phase 2 (9 months post mitigation)** **Summary Statistics** (mg/kg FW) **Median ± SD [range]**
	Blood ((n = 6 x 2 replicates) Liver & Kidney (n = 5 x 4 replicates) Thyroid (n = 4 pooled x 4 replicates)	Liver & Kidney (n = 8 x 4 replicates) Thyroid (n = 6 pooled x 4 replicates)
Br	Blood 44.41 ± 14.88 [27.98-88.19] Liver 13.47 ± 14.01 [6.79-54.1] Kidney 37.66 ± 16.440 [16.47-76.54] Thyroid 16.135 ± 4.269 [15.41-24.38]	Liver 21.28 ± 10.036 [11.99 51.92] Kidney 15.38 ± 24.250 [6.02-85.18] Thyroid 6.56 ± 4.753 [2.37-7.37]
Cu	Blood 1.865 ± 0.206 [1.442-2.091] Liver 28.28 ± 26.02 [7.30-77.2] Kidney 13.08 ± 6.664 [5.97-24.82] Thyroid 0.59 ± 0.106 [0.424-0.676]	Liver 53.72 ± 47.69 [13.2-138] Kidney 17.4 ± 5.789 [6.481-23.87] Thyroid 0.62 ± 0.220 [0.43-0.978]
Se	Blood 0.575 ± 0.122 [0.414-0.941] Liver 1.13 ± 0.30 [0.54-1.62] Kidney 2.52 ± 0.450 [1.56-3.41] Thyroid 0.266 ± 0.065 [0.22-0.373]	Liver 1.103 ± 0.935 [0.793-3.861] Kidney 3.065 ± 0.989 [0.873-3.98] Thyroid 0.278 ± 0.086 [0.188-0.408]
I	Blood 0.09 ± 0.146 [0.02-0.5] Liver 0.01 ± 11.55 [0.01-27.92] Kidney 3.01 ± 2.788 [0.31-9.11] Thyroid 461.3 ± 44.838 [428-532.6]	Liver 0.673 ± 8.941 [0.099-28.08] Kidney 1.144 ± 9.187 [0.084-34.63] Thyroid 312 ± 81.26 [251.1-411.5]
Mn	Blood 0.025 ± 0.009 [0.01- 0.51] Liver 2.27 ± 0.40 [1.49-2.99] Kidney 1.09 ± 0.179 [0.577-1.385] Thyroid 0.207 ± 0.122 [0.122-0.411]	Liver 2.481 ± 0.566 [1.657-3.636] Kidney 1.29 ± 0.505 [0.7-2.449] Thyroid 0.253 ± 0.089 [0.73-0.438]
V	Blood 0.02 ± 0.005 [0.017-0.037] Liver 0.07 ± 0.04 [0.028-0.172] Kidney 0.063 ± 0.01 [0.049-0.087] Thyroid 0.013 ± 0.003 [0.011- 0.02]	Liver 0.137 ± 0.041 [0.093-0.232] Kidney 0.115 ± 0.110 [0.076-0.417] Thyroid 0.017 ± 0.011 [0.012- 0.059]
Cr	Blood 0.145 ± 0.009 [0.116- 0.187] Liver 0.2 ± 0.03 [0.116-0.273] Kidney 0.195 ± 0.072 [0.112-0.67] Thyroid 0.185 ± 0.270 [0.142-0.708]	Liver 0.142 ± 0.061 [0.08-0.353] Kidney 0.106 ± 0.030 [0.061-0.173] Thyroid 0.099 ± 0.032 [0.069- 0.145]
As	Blood 0.105 ± 0.031 [0.066-0.195] Liver 0.07 ± 0.021 [0.029-0.119] Kidney 0.125 ± 0.024 [0.09-0.208] Thyroid 0.050 ± 0.013 [0.038-0.071]	Liver 0.027 ± 0.026 [0.004-0.10] Kidney 0.063 ± 0.032 [0.003- 0.165] Thyroid 0.033 ± 0.014 [0.017-0.068]
Zn	Blood 3.74 ± 1.039 [2.964-7.081] Liver 91.31 ± 25.98 [74.98-177.5] Kidney 27.4 ± 6.940 [19.45 -9.63] Thyroid 13.22 ± 6.352 [9.359-23.36]	Liver 95.83 ± 32.37 [29.78-135] Kidney 32.89 ± 13.35 [21.16-64.39] Thyroid 21.9 ± 6.70 [13.63-28.97]

8.4.2.3 Water quality

The key PHCC noted for all subterranean sources was bromide. The COC strontium occurred with bromide, a trend observed in previous WRC reports. These anomalies are viewed as a combination of naturally occurring geochemical anomalies and cumulative impacts from the use of agricultural chemicals in the general area. Some evidence was noted suggesting that the water resources may be impacted by the intensive agricultural activities in the general area.

8.4.2.4 Tissue values

It is noted in the scientific literature that the use of pigs for biomedical research is hampered by the lack of reference values for common functional variables. Some reference values used are thus best viewed within the context of the observational conditions which may not be entirely reflective of the intensive conditions under which the specimens were obtained. The values should thus be taken to serve as a clinical reference point from which changes over time may be compared to and thus viewed as monitoring inputs. Comparisons by the consultant veterinarian to other piggeries with similar genetics and production inputs should also be considered. Despite some differences between porcine models and humans (e.g. extracellular space, cardiac output, arterial pressure and renal dynamics) they are generally accepted as an excellent model for functional studies relating to human health.

The inorganic chemistry observed is obtained in order to gain insight into trends between tissues and over time. Accordingly, the values should form part of a monitoring programme in order to evaluate the production system in terms of changes effected over time, ranging from nutritional to improved genetics. As such, the values are usually unlikely to present with clear deficiencies or toxicities. In the event of such disturbances additional clinical evidence would be expected to be presented by affected animals. The interpretation of the results is thus usually of value in gaining insight into subclinical chronic disruptions or disturbances in trace element metabolism. Reference range interpretation is thus usually viewed as a probabilistic function and not a deterministic value. As an example, low values in the “deficient” range are thus indicative of a probable improvement on supplementation as opposed to being suggestive of a clinical deficiency.

8.4.2.4.1 TT4 Reference values

Total Thyroxine serum values (TT4) are viewed as of diagnostic value for low ranges indicative of iodine deficiency. This is due to the blood values representing long-term status and not being responsive to short-term fluctuations. Thus, low values are viewed as indicative of potential iodine deficiency disorders, ranging from moderate to severe with primary or secondary causation dependent on a variety of factors.

- Pig Reference Values for Biomedical Research:
    - TT4 (µg/dL) = 2.6 ±0.5 [1.8-3.5]

- Equates to **nmol/L**: **33.46** ±6.43 [23.16-45.09]
- Taken under basal conditions.

- Swine CLA supplementation experiment:
  - Swine: Fatteners:
    - **Lowest** TT4 (nmol/L) for control group (no CLA) = **24.27**
    - **Highest** TT4 (nmol/L) for 0.5% CLA suppl = **39.87**

- Assessment of reference values for different swine categories:
  - TT4 (µg/dL) = 2.6 ±0.5 [1.8-3.5]
  - Equates to **nmol/L**: **52.50** ±7.979 [38.61-64.86]

- Other:
  - Published data for TT4 (nmol/L):
    - Sows: various stages: **34.877-55.46**
    - Sows: 3-4 weeks *postpartum*: *17.17-23.03*
    - Gilts: **68.21-75.93**
    - Weanlings: **102.7-116.2**
    - End 1st fattening stage: **66.92-68.63**
    - End 2nd Fattening stage: **51.22-131.2**
    - Pot-bellied pigs range: 18-73

Human Reference value range (normal TT4 nmol/L):

58-161

(GlobalRPh Lab Values)

8.4.2.4.2 Assessment of Observed TT4 values:

The observed values presented with a median value of 29.6 nmol/L. Compared to the ranges cited above this would appear to be in the deficient band. The range observed of 27-49 nmol/L also places the standard deviation within a narrow concentration range with even the elevated value below the lower range value reported by many workers. Whilst the histopathology did not present with any remarkable evidence, disruption may be at the post-vacuolation stage. This would imply that storage appears adequate even with disruption as the gland attempts to increase the synthesis of thyroid hormones (typical goitrogenic presentation), but the release is dependent on TSH release and response thereto in the follicle. Problems with the subsequent lysosomal proteases affecting colloidal release of MIT, DIT, T3 and T4 may thus occur.

With reference to the tissue inorganic values reported later, it is noted that low thyroid iodine values have been observed in histologically normal thyroid glands. When viewed in conjunction with the low iodine values the interpretation would be that iodine status is marginal and would benefit from supplementation. Since the TSH release is part of a fundamental feedback control system, end-target utilization of the released T4 may also be a factor. *Definitive findings in hypothyroidism of animals are a low T4* (and/or T3) with little or no response to a TSH response test. Increased cholesterol may also be indicated. The relevance of the values may be elucidated when considered in conjunction with the tissue values and possible bromide role in

forming complexes with other essential trace elements. Critically, the response on mitigation chemicals administered approximately for nine months demonstrated that the TT4 values were significantly affected, with ca. 27% increase being observed for Phase 2. The contraction of ca. 40% in the standard deviation of the samples collected also support the observation that thyroid function was stabilizing and not as adversely affected as noted in Phase 1. During the same period the incidence of adverse cardiac events also decreased significantly.

It is important to note that the values obtained are from a small sample pool from the piggery. The recommendation would be to monitor similar categories (sows) at a frequency recommended by the consultant veterinarian in order to assess how representative the values are over time, and to also provide additional information to assist with any differential diagnosis required or to assess benefits with supplementation.

8.4.2.4.3 Liver, Kidney, Whole Blood and Thyroid observations

The following key observations were made (refer to laboratory reports for full results) where:

NR = Normal Range:	Typically reported values.
MS = Marginal Status:	Values *within* this range indicate a probability of a benefit following supplementation. Values *below* this range indicate a significant probability of a beneficial response on supplementation Values *above* this range may benefit with supplementation.
CP = Chronic poisoning:	Values within range indicate high intakes. Values above range indicate definite risk of chronic toxicity

D = Deficiency
M = Marginal
A = Adequate
H = High
T = Toxic

C0 = Control during experimental conditions (0 dietary inclusion level)
CN = Control during experimental conditions (normal dietary inclusion level)
CS = Supplementation benefit achieved during experimental conditions

EDC = Endocrine Disrupting Chemical:
Presence of chemical resulting in disruption/dysfunction of normal endocrine function. May be due to antagonism/synergism/complexes that increase requirements.

NRA No suitable reference available

Table 8.40: Key whole blood observations, with reference ranges and observed median values (mg/L)

Constituent	Range	Observed	Comments
Selenium	0.2	0.575	Normal, marginally elevated.
	2-3 (CP)		Availability questioned.
Copper	1.38 (N)	1.865	Adequate.
	0.19 (D)		May increase with supplementation.
	1.6-2.3 (CS)		
Manganese	0.01-0.012 (MS)	0.025	Above marginal band.
	<0.02 (D)		Deficient value for ruminants.
Bromide	4.1 (N)	44.41	Significantly elevated.
	5.3 (N)		
	6 (H)		
	20 (OEL)		Occupational exposure limit
	30 (OEL)		Indicates hazardous exposure
	96 (T)		Values > 96 indicate brominism.
Iodine	0.022-0.033 (MS)	0.09	Reference for PBI.
			Refer to I:Br ratio comments.
			May reflect adequate diet but EDC.
Zinc	5-15 (N)	3.74	Low to deficient
	2.2 (CO)		Experimental deficiency in pigs.
	8.6 (CS)		Elevated values in replete pigs.
			Lowered values noted in thyroid dysfunction.

Table 8.41: Key kidney observations, with reference ranges and observed median values (mg/L)

Constituent	Range:	Observed	Comments
Selenium	1.5-2.9(A)	2.52	Adequate.
	7.2 (CN)		Normal Se [diet] may yield higher values.
	3.82 (N)		Below Normal pig value.
	16-118 (CP)		Below Normal experimental Control value.
Copper	12-25 (H)	13.08	Adequate to low for High band.
	35-56 (CS)		Supplementation possible.
Manganese	1.56 (N)	1.09	Lower marginal band.
	1.37 (CN)		Lower than normal values.
	1.33 (CS)		
Bromide	RA	37.66	Value higher than liver value.
			Suggestive of reabsorption (long half-life).
Iodine	NRA	3.01	
Zinc	15-30 (A)	27	Within adequate range values.
	42 (N)		Higher normal values observed.
	>200 (H)		Elevated precautionary supply possible

Table 8.42: Key liver observations, with reference ranges and observed median values (mg/L)

Constituent	Range	Observed	Comments
Selenium	0.4-1.2(A)	1.13	Adequate.
	0.141 (D)		Value observed in MHD (Se defic in pigs).
	2.3 (CN)		Normal Se [diet] may yield higher values.
	13-64 (CP)		Observed value below High range.
Copper	15-200 (H)	28.28	Adequate.
	27-308 (CS)		Supplementation possible.
Manganese	2.3 (CN)	2.27	Lower marginal band.
	3.32 (N)		Mean normal values higher.
	2.93 (CN)		Zinc supplementation increased values.
	3.46 (CS)		
Bromide	NRA	13.47	
Iodine	NRA	0.01	
Zinc	40-90 (A)	91.31	Within adequate range values.
	35.6 (D)		
	>200 (H)		Elevated precautionary supply possible.

Table 8.43: Key thyroid observations with reference ranges and observed median values (mg/kg FW):

Constituent	Range	Observed	Comments
Selenium	1.5-2.9(A)	2.52	Adequate.
	7.2 (CN)		Normal Se [diet] may yield higher values.
	3.82 (N)		Below Normal pig value.
	16-118 (CP)		Below Normal experimental Control value.
Copper	12-25 (H)	13.08	Adequate to low for High band.
	35-56 (CS)		Supplementation possible.
Manganese	1.56 (N)	1.09	Lower marginal band.
	1.37 (CN)		Lower than normal values.
	1.33 (CS)		
Bromide	RA	37.66	Value higher than liver value.
			Suggestive of reabsorption (long half-life).
Iodine	NRA	3.01	
Zinc	15-30 (A)	27	Within adequate range values.
	42 (N)		Higher normal values observed.
	>200 (H)		Elevated precautionary supply possible.

8.4.2.4.4 Assessment of Liver, Kidney, Whole Blood and Thyroid observations:

Selenium:

The tissue profile would appear to suggest possible complexes (presumably with Br) that may elevate whole blood, but be unavailable to functioning tissues, yielding low

thyroid and liver values. The kidney value may indicate the complex to have a low tubular transport maximum resulting in a high clearance rate. Values suggest room for additional Se-Supplementation and possible benefit with supplementation. Since Selenium is a component of the thyroid hormones and deficiency thereof is linked to decreased deiodinase activity, the low thyroid Iodine values observed were supportive of these comments. Some researchers report that pig diets may provide suboptimal Vitamin E and Se, notably in piglets due to the low activity of carboxylester hydrolase enzymes in the gut of weanling pigs (these enzymes cleave tocopheryl acetate to form Vitamin E), with the supplementation of diets with non-esterified d-a-tocopherol advocated.

Bromide:

The I:Br ratio returned a value of 28, which is similar to control values observed in experimentation concerning the effects of bromide on iodine and thyroid function. Normally this ratio decreases significantly as the bromide replaces iodine in the thyroid gland. The observation of a low thyroid bromide and iodine whilst whole blood values for both Br and I were elevated would appear to indicate that complexes are forming (presumably hepatic) that are unavailable for thyroid uptake, with the typical effect EDC of bromide on lowering T4 values thus still remaining. Bromide values are usually highest in the liver and kidney, and lowest in whole blood, whilst the observations returned the reverse (whole blood highest, with kidney also ca. three times liver values). These observations would again support the formation of complexes (hepatic phase 1 and phase 2 bio-transformations) that lead to elevated systemic values and renal reabsorption/accumulation.

It is noteworthy that in humans, 22% of patients seen for thyroid disorders present with high plasma bromide values (> 6 mg/L). In many instances TSH is elevated in these patients whilst blood TT4 remains normal. The general adverse effects have been described as:

- Inhibition of I uptake by the thyroid
- Inhibition of the oxidation of iodide to iodine
- Inhibition of the incorporation of iodine into tyrosine residues
- Inhibition of the coupling of tyrosine to thyronine (thus elevating TSH level).

It is also widely reported that females present with elevated values under similar exposure settings to males (mean ± SD: females 5.5 ± 1.5; males 5.1 ± 1.4). The significant effects of bromide exposure on broilers were presented in the WRC Final Report for K5/1915 and should be consulted for further physiological and histopathological detail.

Manganese:

The values were marginally suggestive of a potential benefit on supplementation. Requirements are reported to be significantly greater for pig reproduction compared to growth. Given that deficiencies are not easily distinguished by biochemical manifestations, a precautionary approach would still provide adequate range for supplementation. It is noteworthy that cardiac tissue is more responsive to Mn

deficiency than other tissues and linked to increased cardiac damage (related to reduced MnSOD activity leading to free-radical damage). A Mn-responsive cardiac mitochondrial ultrastructure problem is reported in broilers.

Other elements:

It should be noted that tissue interpretations are not always correlate with supply/availability. For example, zinc values in liver rarely decrease more than 20% even in severe deficiency. Although the nitrate exposure concentrations have been significantly reduced, it should be noted that the guideline value is derived for methemoglobinaemia risk. Nitrate is accepted as an Endocrine Disrupting Chemical, with thyroid function noted as a health end-point. These effects are usually at lower concentrations than those producing methemoglobinaemia. This should be considered as part of a multi-factorial problem and not in isolation with simple bromide exposure.

8.4.2.4.5 General Discussion

Possible link to observed cardiac effects

The effects of copper, selenium and manganese may be contributory to changes in anti-oxidant pathways. Some of the effects are briefly noted below. Selenium deficiency symptoms may occur in conjunction with additional stress (notably vitamin E deficiency) and includes cardiac-myopathy (also a risk factor for peripartum cardiomyopathy), ischemic heart disease and peripheral vascular disease. A fundamental factor involved is lower glutathione peroxidase activity leading to increased lipid peroxidation and oxidative stress levels. In pig models, myocardial infarction was reduced by selenium supplementation, leading to a lowered occurrence of late ventricular potentials in the border zone. Recognition of the smooth muscle relaxant properties have also led to investigation into the use of selenium as an anti-hypertensive agent.

Copper may also be involved in cardiac disorders with deficiency increasing the lipoprotein peroxidation and increased risk of myocyte oxidative damage and consequent elevated plasma cholesterol levels. Long-term copper restriction in rats has led to myofibrillar disarray and mitochondrial fragmentation (cytochrome C oxidase activity decreased). Manganese is also a constituent of antioxidant enzymes (superoxide dismutase and adenylyl cyclase). Lowered levels of manganese superoxide dismutase and glutathione peroxidase are associated with dilated cardiomyopathy.

A combination of these settings may thus be relevant within the water quality results observed, with the TDS value being relatively low. This implies hypo-osmotic effects that initiate elevated cardiac output and arterial pressure to increase renal blood flow and pressure to clear a relative water excess (conserve ECF sodium) may result in more adverse effects given the possible trace element setting noted above. These renal effects would be similar to a pressure-diuresis with potential wash-out and increased renal loss for several trace elements. This would also extend to issues of

splenic venous occlusion (delayed/reduced gastric emptying) and elevated perfusion pressures. Long-term this may lead to the spleen becoming embedded in the omentum and to atrophy. Vessels passing through the connective tissue become occluded (not a torsion, but connective tissue contraction), with repetitive events leading to omental-splenic attachment and regression of the spleen.

It is noteworthy that the incidence of adverse cardiac events declined significantly during the period of administering the mitigation chemicals. The positive response of increased TT4 values has already been noted and the tissue values appeared to also be supportive of the conclusions reached, but a final inclusion thereof was not possible at the time of writing this report.

The tissue values are supportive of the conclusions reached in terms of the mechanisms involved, as evidenced by the ca. 58% increase in liver bromide with concurrent ca. 40% decreases in both renal and thyroid values. It is noteworthy that iodine followed a similar trend in these tissues to bromide. The significant elevation in liver copper is also viewed as a physiologically significant response. The trend observed for manganese, zinc and copper would be interpreted as supportive of a general positive response to the mitigation treatment as noted for the TT4 values. In summary, the mechanisms put forward of thyroid disruption as evidenced by thyroid dysfunction and induced deficiencies appear to be valid and also capable of being alleviated when appropriately managed. The hazards thus posed by potential agricultural chemicals are thus capable of both direct and induced adverse effects, but may also be managed.

8.5 DISCUSSION AND RECOMMENDATIONS

8.5.1 Water quality

Detailed discussions were presented in the deliverables submitted for each sampling phase and the reader is referred thereto for background detail. As a general statement the inorganic water chemistry differed significantly between the three sites with the Letsitele site generally being more stable with less stochastic variation. The specifics of the catchment would suggest that the sampling sites in the Letsitele were not subject to the significant seasonal changes that were noted in the other two sites. The Letsitele site was noted to be a potential control reference site with low suspected adverse impacts, with the Komatipoort site presenting as the site with the greatest potentially hazardous chemical constituents (PHCCs). The Vals and Renoster sites presented with significant seasonal and yearly variation and also intermittent high PHCCs. It was concluded that the sampling frequency for the surface water samples should be increased in order to better establish trends for high and low risk periods.

The Komatipoort samples returned values presenting with more risk than the other sites, with significantly elevated values observed for the borehole samples associated with the broiler collection site, together with the nitrate risk posed by the community borehole in the Letsitele site, would suggest that increased emphasis should be placed on groundwater monitoring. Whilst the compliance monitoring for

Section 21 activities for agricultural uses generally stipulate monthly monitoring, this is usually adjusted to quarterly due to the repeatability of abstraction and discharge dynamics to the intensive agricultural enterprises. Where greater variation is applicable, for example irrigation (being influenced by rainfall events) a frequency greater than quarterly may be advisable.

The elevated constituents noted for the Vals river area were similar to the surface water anomalies reported in previous reports. The physico-chemical properties returned acceptable stable values anticipated with groundwater, but the results for the some samples in the Komatipoort and Middle Vaal area and porcine sentinel sampling area did return some indication of nutrient loading in elevated suspended solids and COD values.

8.5.1.1 Bromide supporting information

The bromide values which exceeded 1 mg/L are indicative of contamination by agricultural chemicals. Whilst bromide is observed in subterranean sources, values typically range from 0.001-0.9 mg/L. Values exceeding 0.1 mg/L have been observed with wildlife and commercial animals presenting with induced deficiencies of essential trace elements and thyroid dysfunction typically responsive to alternative water provision and/or targeted supplementation, as evidenced by the WRC Final Report K5/1915 and the sentinel assessments presented here.

Given the sentinel assessment results (and response on treatment in the porcine sentinel site) it would appear that bromide values in excess of 1 mg/L are considered indicative of possible localized geochemical anomalies and probable contamination from agricultural chemicals (pesticides, etc.) and to pose a **high hazard**.

Given the high intake of water in animals in intensive confined feeding operations during not only confinement (secondary physiological drinking), but also key physiological stages (lactation) and high ambient temperatures (primary physiological drinking), ranges of > 0.1 mg/L may also represent a potential risk, notably for:

- Disruption of **thyroid function** and other endocrine mechanisms, including the reproduction system.
- Increased **requirement for copper**, manganese, selenium and iodine, and possibly fluoride and calcium (site-specific dependent)

The US EPA MCL guideline (0.01 mg/L) is conservative and some guidelines have 3 mg/L as a MPL, and 6 mg/L as a CL, mainly due to the possibility of bromate formation (a carcinogen). Main adverse effects may include weight loss, with most bromide salts considered toxic. Bromide is also reported as a potential goitrogen due to direct competition with iodine (resulting in inhibition of T3 & T4 production) with possible adverse effects including poor growth rates, delayed puberty and endocrine disruption affecting reproductive parameters. Testicular degeneration has been noted in bromide toxicity studies.

An increased requirement for copper and iodine may occur with exposure to concentrations that exceed the guideline limits. Since selenium is also required for iodine activation at the thyroid level, and the concentrations observed for all sites was generally below 0.02mg/L, selenium status should also be assessed to ascertain the dietary inclusion level required. This is also indicated as it is reported in animal nutrition studies in South Africa that some areas are prone to selenium excess and others to selenium deficiency (soil, pasture and forage crops). As neonatal intrathyroidal iodine stores are low, suckling presents the primary source of iodine required for adequate thyroid function. In this regard the blocking effect of bromide can significantly reduce maternal milk iodine values. This is further aggravated by the high water turnover rates of young growing animals. For feedlot type production systems (growers), or animals exposed from weaning to slaughter weight, reproductive effects should be of no consequence. However, thyroid dysfunction would still be an obvious consideration.

Bromine (K / Na) has anti-seizure properties and is also used as an effective trace element for hyperthyroid conditions. The presence of chloride can be beneficial in increasing renal clearance rate of Br, but was not a factor for the samples submitted. Unfavourable effects are usually noted when Br: I ratios are high (hypothyroidism), which was not the case for the samples submitted.

High values are suggestive of groundwater pollution, probably by prior use of Br-compounds in agricultural activities (tobacco & strawberry applications), but may also be related to naturally occurring geochemical anomalies (concentrations greater than 0.05 mg/L are suggestive of industrial contamination). Methyl bromide can diffuse through certain plastics, and bromide-treated soils have been routinely observed to increase the inorganic bromide content of food commodities (leafy vegetables accumulate more without phytotoxic symptoms). Exposure to methyl bromide has been recorded from groundwater following leaching of treated soils (concentrations exceeding 9 mg/L of CH_3Br have been recorded). This occurs as bromide is water-soluble and can thus either be taken up by plants or leached into groundwater. Bromide ion is also routinely used as an aquifer tracer.

Compounds currently receiving research focus at an international level relate to the formation of disinfection by-products (DBPs) that are implicated in endocrine disruption and cancer. Bromide is involved in the formation of DBPs when various forms of disinfection methods are used.

It was noteworthy that some indications of bromide contamination have been observed in the selected sites were the use of disinfection chemicals to treat drinking water appears the suspected source.

A more detailed site-specific assessment is required in order to appreciate the impacts of agricultural chemicals on surface and subterranean water resources, as evidenced by the high value for KGWBH which may be expected given the location (proximity to intensive production crop farming) the elevated value for KRHL (also >1 mg/L) may indicate downstream/flow effects several kilometres away.

It is noteworthy that these high values from the Komatipoort area for bromide were also accompanied by elevated mercury, selenium, iodine and phosphate values that were anomalous and typically observed in polluted groundwater samples. Phosphate values averaged at 7.03, 2.27 and 0.41 mg/L for the Komatipoort, Vals river and Letsitele sites respectively.

Although high strontium values were also generally noted with the elevated bromide values, insufficient reference data is available to assess risk. Complications with both thyroid disruption and fluoride anomalies are also expected (due to interactions between strontium and calcium).

It is also noted that in point of use results are crucial to obtain in performing an assessment, as source values may fail to be reflective of concentrating effects (evaporative/high storage temperatures).

Finally, concern is noted internationally for increased environmental levels of bromide and consequently brominated disinfection by-products from industrial discharges, notably from coal-fired power stations using various emission control treatments (Van Briesen, 2013). Concerns in this regard are detailed in Volume 4 of the WRC EDC Manual on Monitoring and Assessment relating to the Kusile Power Station and related activities.

The recommendation for increased monitoring and regulation of bromide is noted internationally, with detailed reviews of both effluent discharge guidelines and drinking water guidelines required (Van Briesen, 2013).

8.5.1.2 General Statement

As noted in earlier reports the elevated values should prompt the investigation of groundwater quality and use within the sensitive receptor present for each area. It follows that the source, pathway and receptor approach should take cognizance of the water source type utilised, with subterranean sources likely to be more significant in some communities, notably in the Komatipoort area, where none of the sources tested were within the target water quality range and the observed values presented with quality that was not fit for continuous use.

The inclusion of air quality as an exposure route is required to assess the relative quantitative risk posed by a PHCC from water. This is true for all exposure considerations as it reflects the contribution to ingestion and exposure. It may well be that air inhalation of a PHCC (e.g. pesticide or THM) may have a more significant contribution to exposure and higher risk in terms of dynamics and kinetics (absorption and effect) when compared to intake via the drinking water.

For this reason the Komatipoort site was included for the purposes of assessing the risk in a sentinel species from pesticide spraying, with observational benefits including birds (broilers) at all ages from day 1 to day 35 being present throughout the year and the use of open-sided houses with sprayed fields immediately adjacent to the production facility.

The complexities in assessing the effects following exposure to a PHCC are aptly presented in a study on the effects of vanadium on cattle which serves as a recommended reference point for researchers involved in similar studies (Gummow, 2005).

In the broader context the following water sample tests may be recommended for agricultural chemicals:

Type A: Generic Approach

- Inorganic Chemistry
- Microbiological Indicator Organisms

Type B: Source pollution or discharge compliance

- Physico-chemical properties (liquid fraction)
- Pollutant (macro and trace inorganic using ICP-MS) and microbiological classification (microbiological indicator organisms) (liquid fraction)
- Compost test (ARC ISCW) (solids fraction)
- Soil test (trace metals) (ARC ISCW)

Type C: On-site tests

- Disinfection residuals: Typically for free chlorine
- Portable instrument / test strip tests, e.g. pH & nitrate & Electrical Conductivity

Type D: Organic compounds

- Organic Water Quality Constituents: Water Research Commission Report: 1774/1/08 (Burger, AEC & Nel, A, 2008/09/01) from www.wrc.org.za.

Type E: Water for application in EDC Bioassays

- Oestrogenic Assays: Water Research Commission Report: 1816/1/10 (De Jager, Aneck-Hahn, Barnhoorn, Bornman, Pieters, van Wyk and van Zijl) from www.wrc.org.za.
- Androgenic and Anti-androgenic Assays: Water Research Commission Project: K5/1956/Deliverable 6: Chapter 3. R Pieters. From www.wrc.org.za

In the selection of methods and laboratories the detection limits offered need to be sufficiently sensitive. It is strongly advised not to conduct partial testing as this often precludes a meaningful assessment from being performed and results in questions being posed regarding uncertainty in the data gaps that subsequently arise. Since many of the relevant agricultural chemicals are either recognized as, or suspected of being, EDCs, and endocrine disrupting effects are contentious it is best to avoid having significant data gaps. Thus, failing to describe the chemistry adequately can prevent a differential diagnosis from being formulated as interactions are insufficiently described.

The macro element determination was conducted on a full quantitative basis with Anions by Ion Chromatography and Cations by ICP-OES. Trace elements were determined by using ICP-MS (Inductively coupled plasma- mass spectrophotometry) methods, with two general types being available. The first is utilised by the Institute for Soil, Climate and Water (ISCW) at the Agricultural Research Council (ARC) which is a semi-quantitative scan, whist the second is a full quantitative ICP-MS procedure employed by the Pelindaba Analytical Laboratories (PAL) at the South African Nuclear Energy Corporation (NECSA). Cost is the major consideration with the ISCW scan sufficient for most investigations and monitoring programmes, specifically if multiple sample media types are being investigated (soil, biological). The PAL process is recommended for compliance testing and in cases where legal action is being considered. The ISCW is part of the AGRILASA scheme (inter-laboratory testing) and was used for this project.

Lastly, although not a focus point in these water quality assessments it should be noted that usually microbiological assessments may be performed by the Food & Microbiological Section at the South African Bureau of Standards in accordance with SANS 241:2006 (Edition 6.1) (SANS 5221: 2007 Ed 4.3 & K.M.5.4W-B-TC & EC) with samples collected on site and delivered to the SABS for pre-arranged processing.

Should any concern be noted regarding the management and possible contamination of the available water sources by agricultural activities or enterprises then the relevant indicator organisms may be determined according to SANS 241:2011 Edition 1. Should the nitrate, phosphate or potassium values from the inorganic chemistry tests indicate a high probability of contamination with agricultural chemicals then the microbiological quality tests would also be indicated.

8.5.2 Sentinel Monitoring

8.5.2.1 Poultry

Significant sample loss occurred during storage of the samples (and possibly handling) with excessive *in vitro* hemolysis noted for most of the Letsitele samples collected, and marginal grades noted for the Komatipoort samples. This was apparently attributed to anti-coagulant properties (tube-product related), but also potentially due to improper mixing, excessive suction (sample collection), storage period (and temperatures). Following discussions with the pathologist the methodology is to be changed to the use of serum TT4 in future using clot activators and horizontal storage after sampling to achieve adequate clot size in the sample volume.

What is evident in the sample sites is that within the perspective of the commercial Ross 308 specimens collected from the Komatipoort site the blood values returned observations for TT4 that were similar to those observed for WRC Project K5/1915 with an average of 5.3 nmol/L and 4.95 nmol/L respectively. These indicated significant thyroid disruption supported by the histopathological observations (refer to WRC K5/1915 Final Report by Meyer *et al*., 2014). By comparison these values are both ca. 50% of the normal reference values obtained from Ross 308 specimens from control sites and literature values for commercial breeds.

The histopathology for the Komatipoort site again returned observations of minimal vacuolation, a key point in the suspected potential role of bromide and nitrate EDC effects on thyroid tissue. It is insightful to note that at the stage of collection the Komatipoort broilers are at the genetic peak of the growth curve and should present with normal to very active vacuolation. The sampling time was also different for the Komatipoort site being at the beginning of the rainfall season (WRC Final Report K5/1915 sampled end summer). Given the variation observed in the catchment for the EDC bioassays and water chemistry, and potential pesticide spraying schedule, it is reasonable to assume a seasonal variation will be evident.

The Letsitele values are more difficult to interpret due to the variation in indigenous breeds, the lack of similar health care provided (body condition poorer) and mature age. As is reported in WRC Project K5/1915 plasma thyroxine values for various strains and ages of broilers differed with the modern broiler strains slightly hypothyroid when compared to the Naked Neck strain (indigenous breed) and concluded that the selection pressures for growth and efficiency may have led to altered thyroid hormone metabolism. Some functional hypothyroidism may be indicated by low T3 and unchanged T4, but in the case of the Naked Neck strain higher circulating T3 and lower T4 values were found at similar ages, supposedly due to the “slightly hyperthyroid nature” in this strain when compared to commercial strains. This accorded with the results obtained from the sentinel specimens in the Letsitele rural setting compared to commercial breed from Komatipoort. Thus, with rural breeds reported to present with the lower plasma T4 values, the reverse observation supported the stance that the comparatively lower commercial breed values noted for the Komatipoort area should be viewed as not only suggestive of and endocrine disruptive effect on thyroid function but also a physiologically

significant event that warrants further investigation into public health within groundwater users in the area.

The combination of EDC exposure media data, blood hormone values and histopathology are thus recommended to contribute towards confidence in both diagnostics and the decision to accord priority for further investigation to specific sites. The recommendation at this juncture would be to obtain serum samples using the new method and to first obtain additional rural specimens to achieve sufficient reference values between local indigenous breeds and across seasons for the sample sites. The porcine sentinel sampling results would support this approach.

The observation in late 2014 of feminization in cockerels at the Komatipoort site was suspected with immature testes having been observed previously (Meyer *et al*., 2014). Late 2014 broiler sentinel sample results were not yet available at the time of writing this report. It was also a recommendation to obtain poultry sentinel samples from the Renoster and Vals river areas but due to project constraints this was not performed.

8.5.2.2 Porcine

The tissue observations would suggest increased requirements for Cu, I, Se, Mn and Zn to be applicable. Those observed values that were within adequate ranges tended to occur within the lower zones of the bands. The values were also for many observations lower than those reported in supplemented intensive pigs, and significantly below values associated with chronic excesses. Accordingly, a precautionary approach of increasing these elements was followed (increasing dietary supply without high risk of chronic excess).

In order to improve thyroid function reducing the bromide exposure would be preferable, yet without the availability of an alternative low bromide water resource, reverse osmosis is the only other effective treatment. This treatment option is unfortunately not financially viable for commercial operations with high water requirements. A physiological treatment was instead adopted to increase the renal clearance rate of bromide and so reduce the adverse pharmacodynamics effects noted, whilst simultaneously alleviating the hypo-osmotic risks inherent in the untreated drinking water.

This administration of mitigation chemicals to the drinking water supply and dietary trace element formulation adjustment appears to have significantly alleviated the adverse cardiac and thyroid events. This is confirmed by routine veterinary examination of the breeding animals and by subsequent increased TT4 levels.

8.5.3 Endocrine aspects of Thyroid Function

Extensive publications exist on disorders involving the thyroid gland in humans making thyroid function possibly the most commonly reported endocrine disorder. Nutritional disorders involving iodine and thyroid function are also well documented.

An anatomical consideration regarding thyroid function relates to the fact that it is a highly vascularized tissue. Although the functional unit is considered to be the thyroid follicle (the source of thyroid hormones), interspersed are also thyroid C cells which produce the hypocalcemic hormone Calcitonin. Parathyroid glands are often considered as a third type of thyroid tissue as they may be imbedded in the thyroid gland in some species (or located in close proximity) and produce parathormone (a hypercalcemic hormone).

What makes the thyroid gland so relevant to water quality is that iodine is an integral part of its hormone, thyroxine (T4). Selenium is also required for the activation of iodine for its inclusion into T4. Since iodine is required the occurrence of iodine-deficiency disorders is well-documented. In addition the thyroid gland is different to most endocrine glands in that it is capable of storing large quantities of hormone with the iodine content a significant proportion of its weight. The principal hormones elaborated by the thyroid are T4 (3,5,3',5'-tetraiodothyronine), T3 (3, 5, 3'-triiodothyronine), and rT3 (reverse T3), with T3 being the active form in the target cell and T4 the transport form and feedback regulator.

The compensatory hypertrophy of thyroid follicular cells is well-documented and results in the development of iodine deficiency goiters (enlargements of the thyroid gland). Some chemicals may be disruptive by blocking the thyroid trapping of iodine (which is normally stimulated by TSH). Thyroid hormone has numerous effects, including clinical, developmental to reproductive, and as such is involved in numerous metabolic processes. Goiter is regarded as an enlargement of the thyroid gland that is not due to inflammation or malignancy and may be divided into nontoxic (simple or hypothyroid) and toxic (hyperthyroid). Deficiency or defect at any of the trophic steps may result in thyroid disease, with goitrogenic chemicals blocking the hormonogenic pathways.

Complications in the diagnosis of thyroid dysfunction are the ability of the gland to undergo hypertrophy and hyperplasia to increase uptake and secretion so that normal hormone levels are maintained despite visible increases in thyroid mass.

Lastly, it is noted that whilst the focus on EDCs (and agricultural chemicals suspected of having EDC effects) has focused initially primarily on the reproductive axis, more priority is being assigned to those involving non-reproductive endpoints. A thyroid-bioassay is being developed in a separate WRC project and should be a meaningful addition to the WRC Manual on EDC Toolbox. The use of broilers and porcine samples for histopathology and clinical biochemistry is still argued as being a valuable site-specific assessment tool, as demonstrated by the findings reported in this Chapter.

8.5.4 Wastewater from Agricultural Water Use Activities

It is noted that whilst significant changes were effected to Acts affecting the handling of wastewater generated by agricultural activities during the project period, effective handling and interpretation thereof was not evident in the authorisations and licences granted.

It follows that in order for the hazards and risks posed to not only be observed, but also managed, appropriate monitoring and therefore inclusion of the relevant agricultural chemicals must be conducted to a greater extent.

8.5.4.1 Relevant Legislation

It is apparent that increased management of agricultural chemicals in water resources is required from the Department of Water and Sanitation given the following recent Government Notices which:

- Permit the application of wastewater for irrigation purposes as contemplated by the authorized activity as a General Authorisation thereby removing the need for a Water Use Licence; and
- Have amended the list of activities that may have a detrimental effect on the environment by deleting those dealing with the storage, treatment or processing of animal manure, and the treatment of effluent, wastewater or sewage, thereby removing their need for a Waste Management Licence.

The recent relevant Government Notices are:

- **Government Notice No. 665 of 6 September 2013** (National Water Act. Act 36 of 1998)
- **Government Notice No. 921 of 29 November 2013** (National Environmental Management: Waste Act. Act 59 of 2008)
- **Government Notice No. R 922 of 29 November 2013** (National Environmental Management Act: Act 107 of 1998)

Critically the Government Notice No. 665 of September 2013 notes that:

- Schedule 1 [Section 21 (e)]: section 1.1 of the Schedule notes: "This Schedule ***replaces the need for a water user to apply for a licence*** in terms of this Act, provided that the water use is within the limits and conditions as set out in this general authorization."
- This detail as presented in Section 21 (e) Controlled Water Use Activity thus permits the application of biodegradable wastewater, such as that generated by an intensive confined animal feeding operation, to agricultural land as part of a general authorization, on the provision that the activity complies with the conditions stipulated.

The Government Notice No. 921 of 29 November 2013 notes that:

- The National Environmental Management: Waste Act, 2008 (Act 59 of 2008): List of waste management activities that have, or are likely to have a detrimental effect on the environment, as published under Government Notice 718 in Government Gazette 32368 of 3 July 2009, in terms of schedule 19(2)

of the National Environmental Management: Waste Act, 2008 (Act 59 of 2008), are amended in the schedule thereto.

- As proposed in the amendments to the Government Notice 718 of 9 July 2009**:** National Environmental Management: Waste Act (59/2008): List of waste management activities that have, or are likely to have a detrimental effect on the environment, detailed in the GN 779 of 28 September 2012 proposal:
    - For both Category A and Category B activities:
        - Excluded the animal manure description (Activity 17 of GN 718)
        - Stipulated "the exclusion of effluent, wastewater or sewage" from the required list (Category A (6) and (B (4)).
    - This was promulgated as the current GN 921 of 29 November 2013 and is *thus applicable*.
- The removal of these listed activities is in line with the recognition that the activities pertaining to animal wastewater are appropriately accommodated by the GN 665 of 6 September 2013.
- As was noted above references to animal manure, effluent, sewage and wastewater have been removed from these listed activities. References are made to general waste (Category A) and hazardous waste (Category B), and it is noteworthy that animal wastewater generated is neither general nor hazardous waste, but as per the definitions presented in the GN 665 of 6 September 2013, the animal manure with added water complies with the "biodegradable industrial wastewater" and is defined under section 1.6 " Definitions" of the Schedule thereto as:
    - "wastewater that contains predominantly organic waste arising from industrial activities and premises including:
        - (j) confined animal feeding operations.

The Government Notice No. R 922 of 29 November 2013 notes that:

- The National Environmental Management Act, 1998 (Act 107 of 2008): Amendments to Environmental Impact Assessment Regulations Listing Notice 1 of 2010, Published under Government Notice R 544 in Government Gazette 33306 of 118 June 2010, as amended by Government Notices R660 in Government Gazette 33411 of 30 July 2010 and R1159 in Government Gazette 33842 of 10 December 2010 , under section 47A (1)(b) read with section 24(2), 24(5) and 24D of The National Environmental Management Act, 1998 (Act 107 of 2008), are amended in the schedule thereto.
- The following is noted in section 2 with reference to the Insertion of Item 55A in the Notice:
    - 2. The following items are hereby inserted in the notice:
    - Activity 55A of Listing Notice 1:

        - 55A: “The construction of facilities for the treatment of effluent, wastewater or sewage with a daily throughput capacity of more than 2000 cubic metres but less than 15 000 cubic metres.”

- It should be noted that this minimum throughput volume applies to:
    - Municipal wastewater treatment plants
    - And does not apply to a commercial confined animal feeding operations, as:
        - A large commercial animal production facility will typically generate ca. 50 cubic meters of wastewater per day
        - Farms with multiple production sites will seldom exceed a combined wastewater production of more than 100 cubic meters per day.
        - This is significantly less than the 2000 cubic meter minimum volume stipulated in Listing 55A.
- It thus follows that no commercial animal operation in South Africa would trigger the Listed Activity as amended in the GN R 922 of 29 November 2013.

The Government Notice No. 332 of 2 May 2014 notes that:

- The activity listed under Category A 3 (8) is deleted from the list.
- This activity refers to the Remediation of Contaminated Land, and is accordingly not relevant to animal wastewater.
- It is thus apparent that the recent amendments to the GN 921 of 29 November 2013 have not altered the removal of the listed activities dealing with animal manure and wastewater, and their deletion from the Listed Activities remains.

The recent revision in terms of Section 39 of the National Water Act, 1998 (Act 38 of 1998) as presented in **Government Notice No. 665 of 06 September 2013**, of the General Authorisation in Section 3 of the schedule to Government Notice No. 398 of 26 March 2004, and the general authorisations in sections 2, 3, and 4 of the schedule to Government Notice No. 399 of 26 March 2004, refers.

Schedule 1 [Section 21 (e)]

Schedule 1

1. Engaging in a controlled activity, identified as such in Section 37(1): Irrigation of any land with waste or water containing waste generated through any industrial activity or by a waterwork.
   [Section 21 (e)]

Section 1.1 of the Schedule notes: “This Schedule ***replaces the need for a water user to apply for a licence*** in terms of this Act, provided that the water use is within the limits and conditions as set out in this general authorization.”

Definitions

1.6

"**Biodegradable Industrial Wastewater**" means wastewater that contains predominantly organic waste arising from industrial activities and premises including:

(j) confined animal feeding operations;

"**irrigation**" means the application of wastewater to any land or property for the purpose of crop production, and includes the cultivation of pasture or any other suitable purpose;

"**organic waste**" means waste of non-anthropogenic origin that is readily biodegradable in the environment and does not contain any toxic substances that may accumulate in the environment;

Section 1.7 of the Schedule notes:

"A person who-

- Owns or lawfully occupies property…
- Lawfully occupies or uses the land…
- lawfully has access to land on which the use of water takes place,

may on that property or land

(i) irrigate up to 2000 cubic meters of domestic and biodegradable industrial wastewater on any given day as set out in Table 1.1:

(iv) irrigate provided that the irrigated wastewater does not impact on a water resource or any other person's water use, property or land; and

(v) irrigate provide that the irrigated wastewater is not detrimental to the health and safety of the public in the vicinity of the activity.

Precautionary Practices

Section 1.10 (1) of the Schedule notes:

"The water user must follow acceptable construction, maintenance and operational practices to ensure the consistent, effective and safe performance of the wastewater irrigation system, …"

Section 1.10 (2) of the Schedule notes:

"Suspended Solids must be removed from wastewater, and the resulting sludge disposed of according to the requirements of any relevant law or regulation, including Guidelines for the Utilisation and Disposal of Wastewater Sludge, Volumes 1-5,

Water Research Commission Reports TT 261/06, 262/06, 349/09, 350/09, 351/09, as amended from time to time."

Schedule 3 [Section 21 (g)]

Schedule 3

3. Disposing of waste in a manner which may detrimentally impact on a water resource.

[Section 21 (g)]

Section 3.6 presents the same definitions as noted for Schedule 1.

Section 3.9 permits the user to dispose of biodegradable industrial wastewater to wastewater pond or evaporation systems with specific volumes stipulated.

Section 3.16 (5) (a) again refers to the Guidelines for the Utilisation and Disposal of Wastewater Sludge, Volumes 1-5, Water Research Commission Reports TT 261/06, 262/06, 349/09, 350/09, 351/09.

South African Sludge Classification System

The Guidelines for the Utilisation and Disposal of Wastewater Sludge, Volumes 1-5, Water Research Commission Reports TT, to which the GN 665 of 6 September 2013 directs the user to refer. The appropriate volume applicable to animal wastewater is Volume 2 of 5: Requirements for the Agricultural Use of Wastewater Sludge (WRC TT 262/06). The permissible application of wastewater sludge from animal production facilities to agricultural land is described in great detail by this Volume. It is relevant to note that such activities are not only comprehensively dealt with by the NWA and relevant Government Notices, but advocated by the **Minister of Water Affairs** in this Volume 2 guideline documentation as:

"…specifically developed to encourage the responsible use of wastewater sludge in agricultural practices. The agricultural use of sludge is defined as the beneficial use….to **benefit either the soil condition and/or enhance crop production in a sustainable manner**. **The potential benefits of the nutrients….have been well demonstrated and have led to the utilization of sludge for agricultural practices in many countries. The agricultural use of sludge is seen as an appropriate cost effective management option for South Africa both for the agricultural and wastewater industry**." (WRC, TT 262/06).

8.5.4.2 Summary Statement

In closing it is argued that for these schedules and applicable precautionary practices to be applied, the recognition of the role played by the competent authorities in stipulating and enforcing compliance requires due regard for the agricultural chemicals that may be applicable.

Whilst this may not be practical for each agricultural activity, the following general approach should be considered as a fundamental stipulated requirement for both water resources and wastewater (raw and liquid fractions):

- Inorganic Chemistry (macro and ICP-MS for trace elements, including Br)
- Physico-chemical (Chemical Oxygen Demand, Suspended Solids, Surfactants, Ammonia)
- Microbiological Indicator Organisms (Total Bacteria Count, Total Coliform Count, Faecal Coliforms and *E. coli*).

Additional constituents and parameters may be applicable to receiving environments and specific agricultural activities and could thus be additional monitoring requirements.

Lastly, it is also clearly in the interests of the agricultural sector to increase the compliance monitoring as by implication they are also protecting the fundamental water resource input required for a sustainable economic environment, as opposed to only monitoring as a reactive measure to investigate adverse effects associated with impacts on water resources.

8.6 CONCLUSIONS

The key research themes requiring elucidation include:

- Further investigation is needed to identify the sources of bromide, both from naturally occurring geochemical anomalies and agricultural chemicals.
    - Significant increases have been noted when various disinfection chemicals have been used in drinking water applications for both surface and groundwater in agricultural water use activities.
    - Further investigation is indicated to establish the key adverse effects linked to bromide. Whilst the thyroid disruption and dysfunction appears to be a consistent observation in broilers and porcine sentinel monitoring, additional reproductive and induced deficiencies require more focused research.

- The use of sentinel monitoring has yielded valuable information towards the site-specific animal health assessment. The observations would suggest that further studies into human health with bromide as a key priority chemical are indicated.
- The inclusion of the full ICP-MS suite of chemicals, with the notable inclusion of bromide, for both water resource compliance monitoring, and the supporting tissue tests as conducted for the sentinel monitoring, is justified and should be part of compliance monitoring for Section 21 activities of the National Water Act.
- In order to interpret the hazards and risks posed by agricultural chemicals background baseline exposure to inorganic chemistry is required as a starting

point. Failure to include this results in less confidence in interpreting both the bioassay results that may be obtained and exposure assessments. Use of this water quality data is required in order to meaningfully interpret the context of hazards posed by organics and inorganics, without which a differential diagnosis may be difficult to reach. This is presented in greater detail in the Monitoring and Assessment Volume of the WRC Manual on EDCs.

- An increased frequency of observations is required for the bioassays and suspected organic hazards (e.g. pesticides). It would appear that a low hazard was noted for the organics in the selected sites, this possibly being a function of the hazard itself being transient in nature. It may be argued that the surface water recharge in the selected sites reduced the potential for risk, but this may not be true for other areas or groundwater, and these should accordingly be a focus point of any site-specific investigation.
- In order to manage agricultural chemicals effectively it should be noted that the source may be varied to a great extent, and include constituents included in animal feeds, washing, sanitizing and disinfection chemicals. It follows that in order to effectively comply with the objectives of the applicable legislation, the beneficial reuse of wastewater can only be conducted if the appropriate monitoring thereof is conducted. It was an observation during this project that many authorizations and licences granted failed to include the constituents relevant to the process involved. In terms of confined animal feeding operations the dual use of anaerobic digestion and a separator stage may assist in reducing the adverse effects linked to health parameters in receiving receptors. This is a growing and complex issue which also warrants further research.
- It is also acknowledged that a revision of the 1996 South African Water Quality Guidelines is underway with the irrigation volume being addressed first. It is argued that both Domestic and Animal Watering sections also urgently require revision to align with risk-based approaches that are necessary to appropriately assess and manage the hazards and risks present.
- In the interim a position statement for competent authorities currently administering both applicable sections of the NEMA and NWA should be prepared and submitted as an outcome of this project.

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CHAPTER 9: HUMAN HEALTH RISK ASSESSMENT

by

B. Genthe and J. Chamier

CSIR, Natural Resources and Environment, PO Box 320, Stellenbosch, 7599

9.1 INTRODUCTION

South Africa is one of the major users of pesticides on the African continent (Heeren et al., 2003). Currently, South Africa has more than 500 registered pesticides (PAN, 2010) and is one of the four largest importers of pesticides in sub-Saharan Africa (Osbanjo et al., 2002). In 2006 the import of insecticides, fungicides and herbicides that were packaged for retail totalled $ 170 056 000 the main import partners being Australia, China, Germany and the United States of America (USA) (International Trade Centre, 2011). Concerns have been expressed that some of the pesticides used in agricultural practice may enter and pollute rivers and dams and cause endocrine disrupting effects in animals and humans that use the water for drinking, irrigation and recreational purposes. To-date there has not been clarity on the extent and levels of contamination of water resources by agricultural products, especially those with endocrine disrupting (ED) properties. A number of studies have examined the occurrence of a variety of pesticides in water and the environment and have been found spread throughout the country.

Endocrine disruptors (EDCs) may interfere with the body's endocrine system and cause adverse developmental, reproductive, neurological, cardiovascular, metabolic and immune system effects in humans. Chemical exposure during early stages of development can disrupt normal development and thus drastically alter disease susceptibility later in life (Thaddeus et al., 2011). In general the concern with EDCs is mostly focused on those substances that cause endocrine mediated adverse effects at exposure levels lower than those which cause other adverse effects (WHO, 2002). While the World Health Organization points out that the possibility of adverse outcomes is plausible, they also note that there is a great deal of uncertainty (and controversy) in establishing the link between EDCs at very low environmental concentrations and specific adverse human health effects (WHO, 2002).

Of the EDCs, exposure to pesticides with possible endocrine activity is of particular concern as these chemicals are deliberately released into the environment, and given their largely persistent nature, affects can be experienced far from the original source. Due to their persistent nature, pesticides often accumulate in the food chain and people are generally exposed through the consumption of fruit and vegetables, contaminated meat, fish, and dairy produce and, in some cases, water.

The situation is more complicated than the risks expected from single pesticide spraying events. While most pesticides with potential endocrine activity are only weakly oestrogenic compared to natural and synthetic oestrogens, in natural waters they occur alongside other EDCs such as alkylphenolics used in detergents (Colborn and Clement 1992). The major concern is therefore with the potential interactive effects of the numerous hormone disrupting substances to which humans and wildlife are now exposed. Undertaking risk assessments

on single substances will not be able to describe the real world situation accurately. It could certainly be envisaged that exposure to a broad range of oestrogen mimicking substances, anti-androgenic substances, substances which inhibit the formation of steroids, and substances which increase their clearance, could give rise to an enhanced de-masculinising effect.

A common ecotoxicological effect of most pesticides is their ability to be bio-magnified (or bio-accumulated) in food webs. This bio-concentration occurs when a compound has a higher concentration in the tissues of an organism, than in its surrounding environment. This often occurs in aquatic organisms but is not limited to aquatic environments, and may also occur in terrestrial species.

A literature review was conducted to describe the general health concerns associated with the pesticides detected in the study sites and to determine the endocrine activity reported. Studies with regards to the possible endocrine health effects of specific pesticides are increasing, but are generally limited to impacts on first and second generations. Investigated concentration ranges also vary, and are generally in comparable regions for established no observed adverse effect level (NOAELS) for the individual pesticides. Very few studies attempt to evaluate multiple exposure effects stretching multiple generations.

This chapter integrates pesticide monitoring data (Chapters 3 and 4) and associated ED bioassay results, together with estimates of exposure pathways to assess the risks of pesticides to human health. Details on the study area and sampling sites can be viewed in Chapter 2.

9.2 METHODOLOGY

9.2.1 Health risk assessment

The methodology with which the human health risk assessment was done is described by the US Environmental Protection Agency (US EPA, 1987; 1992) and the World Health Organization (WHO, 2010). The health risk assessment is primarily divided into four steps: hazard identification, dose response assessment, exposure assessment and risk characterization.

For non-carcinogenic toxic effects of organic pollutants, a Hazard Quotient (HQ) is calculated by comparing the expected exposure to the agent to an exposure that was assumed not to be associated with toxic effects. For the oral exposure of humans to the consumption of contaminated water, meat and plants, the Average Daily Dose (ADD), determined from the measured concentrations, was compared to a Reference Dose (RfD) as reported by the EPA. An HQ <1 was considered to be safe for a lifetime of exposure.

The average daily dose and hazard quotient are determined with equations (1) and (2) with the exposure risk parameters. Many risk parameters can be used to describe a targeted or vulnerable group and are discussed in detail in US EPA (1997; 2002a; 2005). In situations where very little exposure information is available, the average parameters used for baseline human health risk assessment are given in Table 9.1.

Table 9.1: Average exposure parameters used for human health risk calculations

Exposure parameter	Quantity
Events per year	350
Body weight	70 kg
Lifetime	70 years
Ingestion rate	Water: 1 L/day Fish: 54 g/day Vegetables: 250g/day Meat: 40 g/day Water from recreational use: 0.05 L/day

For *non-cancer risks (HQ),* the ADD received during the period of exposure was calculated as follows:

$$ADD = \frac{C(m) \times IR}{BW} \quad (1)$$

$$HQ = \frac{ADD}{RfD} \quad (2)$$

Where:
HQ *= Hazard Quotient*
ADD *= Average Daily Dose ($mg^{-1}\ kg^{-1}\ day^{-1}$)*
RfD *= Reference Dose*
C(m) *= Contaminant concentration ($mg\ kg^{-1}$)*
IR *= Ingestion rate ($kg\ day^{-1}$)*
BW *= Body Weight (kg)*

For the *risk of carcinogens* for exposures that last less than a lifetime (LADD), exposure is calculated as:

$$LADD = ADD \times \frac{ED}{Lft} \quad (3)$$

$$Risk = 1 - e^{-(\beta \times LADD)} \quad (4)$$

Where:

ED = *Exposure duration (years)*
L_{ft} = *Lifetime (years)*
β = oral potency factor (US EPA 2011)

Equation 4 was simplified and can be approximated using Equation 5:

$$Risk = LADD \times \beta \quad (5)$$

The risk estimates represent the theoretical excess cancer risk. This is the risk of developing cancer in addition to the background cancer incidence. For example, if the cancer risk is found to be 1 $x10^{-5}$ = 0.00001 = 1/100 000, then it can be said that there is an excess risk of developing cancer of 1 in a hundred thousand.

The WHO (2011) defines the acceptable risk level as “an estimated upper-bound excess

lifetime cancer risk of one additional cancer per 100 000 of the population ingesting drinking water containing the substance at the set guideline value for 70 years (life expectancy)". Thus, the WHO (2004) and various countries world-wide have set their acceptable risk level at 10^{-5}.

Different exposure scenarios were used to predict possible human health risks, including a person making use of the surface water for their domestic use, a farmer using the water to irrigate produce, a person eating fish from the area and a person making use of the water for recreational activities such as swimming. Modelled air distributions and depositions were also used to calculate potential risks based on spray-drift of applied pesticides. The detailed parameters are presented in appendix 3. The Risk Assessment Information System (© 2013 The University of Tennessee) was used to calculate the average dose making use of the trans-media calculations (described in the next section) to predict the transfer of a chemical from water or sediments to food. Risk Assistant™ (Thistle Publishers, 1993) was used to calculate average inhalation doses. As no Reference Concentrations were available for the modelled air pesticide data Threshold Level Values (TLVs) used for Occupational Health workers (Toxnet, 2010) were used in the risk calculations.

9.2.2 Trans-media calculations

To determine various exposure scenarios to the pesticide of concern, the concentration of the contaminant in water or sediment needed to be converted to another medium. The equations used to calculate the expected average daily doses (ADD) of the pesticides in vegetables, beef, milk and fish through exposure to water are listed in Table 9.2. From these ADDs, the possible human health risk through ingestion of media exposed to contaminated water could be determined.

Table 9.2: The equations required to determine the exposure concentrations in trans-media transfer.

Trans-media	Equation
Water to produce (vegetables and fruit)	$ADD\ (produce) = \frac{Cw \times EF \times IRP \times ED \times CF \times Irr}{ED \times 365\ days/year \times BW}$
Water to beef	$ADD\ (beef) = \frac{Cw \times EF \times IRB \times ED \times CF \times Fb \times Qw\ (beef)}{ED \times 365\ days/year \times BW}$
Water to milk	$ADD\ (milk) = \frac{Cw \times EF \times IRM \times ED \times CF \times Fm \times Qw\ (milk)}{ED \times 365\ days/year \times BW}$
Water to fish	$ADD\ (fish) = \frac{Cw \times EF \times IRF \times ED \times CF \times BCF}{ED \times 365\ days/year \times BW}$

9.2.3 Risk Assessment of Endocrine Disruption

There is no standardised method or guideline to assess human health risks associated with endocrine disrupting chemicals (WHO, 2004). However, an approach similar to toxic equivalency factors is used for hormones and their activity in water, and can be expressed in terms of equivalency factors or quotients (e.g. estradiol equivalent quantity or EEq). A value above the EEq, a so-called trigger value, serves as an indicator that more detailed EDC testing is required. A trigger value of 0.7 ng/L, based on the acceptable daily intake (ADI) of 200 pg/kg/d (as opposed to 50 ng/kg/d suggested by the WHO (2004) to compensate for sensitive subpopulations, individual variation, percentage availability and a safety factor of 1000 was suggested by Genthe *et al.*(2009).

The androgen activity measured in the study could not be used in a quantitative health risk assessment, as a safe environmental level, or level where no androgenic effects are anticipated in either humans or animals is not available at this stage. However, comparisons can be made between the sites where more (anti-)androgenic activity was measured and water quality at those sites.

Water samples may comprise a complex mixture of chemicals with possible (anti)-androgenic and (anti)-oestrogenic activity, as well as other chemicals not measured, that could affect the outcome of the assay. It should therefore be noted that EEqs are only rough estimates, as the complexity of the sample, pH, extraction procedure and the nature of the assay (i.e. a biological system) might all have an influence on the results.

9.2.4 Uncertainty Analysis

Uncertainty analysis was performed using Monte Carlo simulations. The goal of Monte Carlo analyses is to characterise and describe the uncertainty in exposure and risks. Uncertainty analyses provide confidence statements for the estimate of risk. Pesticide data for each study site and each pesticide detected were analysed to determine the distribution of the data. The Excel add-on @Risk (2013, Version 6, Palisade Corporation) was used to carry out the Monte Carlo analysis running 10,000 simulations to determine the range and probabilities of the outcomes.

9.3 RESULTS AND DISCUSSION

9.3.1 Pesticide detection

The average and maximum concentrations of pesticides for the environmental samples were calculated to use in the human health risk assessment and are provided in the table below (Table 9.3). The distribution of pesticides detected at each of the study sites was determined to provide input into the uncertainty analysis of the risk characterisation. The distributions were primarily exponential, with an example of results obtained for atrazine in the Vals – Renoster dataset using @Risk in Figure 9.1 below. This illustrates the measured and predicted concentrations of atrazine making use of Monte Carlo simulations showing that 90% of values can be expected to range between 0.0059 and 0.275 µg/L.

Table 9.3: "Reasonable Maximum" (95th percentiles) and average (ave) pesticide concentrations detected in water (µg/L) and sediment (µg/kg) samples at the study sites

Letsitele		**Diphenyl amine**	**Imizalil**	**Thiabendazole**	**Imidacloprid**	**Propiconazole**	**Androgen**	**Oestrogen**
Water	*95th*	0.00930	0.06189	0.00416	0.25402	0.00398	1.33900	0.41550
	ave	0.00194	0.02694	0.00194	0.09679	0.00057	0.14500	0.11163
Sediment	*95th*	0.00128	0.00000	0.00043	0.00000	0.00020	1.65800	14.19650
	ave	0.00027	0.00000	0.00016	0.00000	0.00004	0.29822	3.50273
Lomati		**Atrazine**	**Imidacloprid**	**Terbuthylazine**	**Thiabend azole**	**Carbofuran**	**Androgen**	**Oestrogen**
Water	*95th*	0.06719	0.04240	0.00991	0.00289	0.00637	1.32500	0.40200
	ave	0.01481	0.00723	0.00315	0.00042	0.00277	0.18571	0.15260
Sediment	*95th*	0.00105	0.00000	0.00047	0.00022	0.00011	1.62650	30.97700
	ave	0.00041	0.00000	0.00025	0.00004	0.00002	0.30375	8.65913
Vals & Renoster		**Alachlor**	**Atrazine**	**Imidacloprid**	**Simazine**	**Terbuthylazine**	**Androgen**	**Oestrogen**
Water	*95th*	0.00200	0.24506	0.00000	0.34830	0.15170	0.00000	2.77000
	ave	0.00034	0.06960	0.00073	0.08239	0.05022	0.05417	0.69831
Sediment	*95th*	0.00000	0.00128	0.00000	0.00100	0.00285	0.97750	167.6490
	ave	0.00007	0.00057	0.00000	0.00017	0.00119	0.10875	32.74435

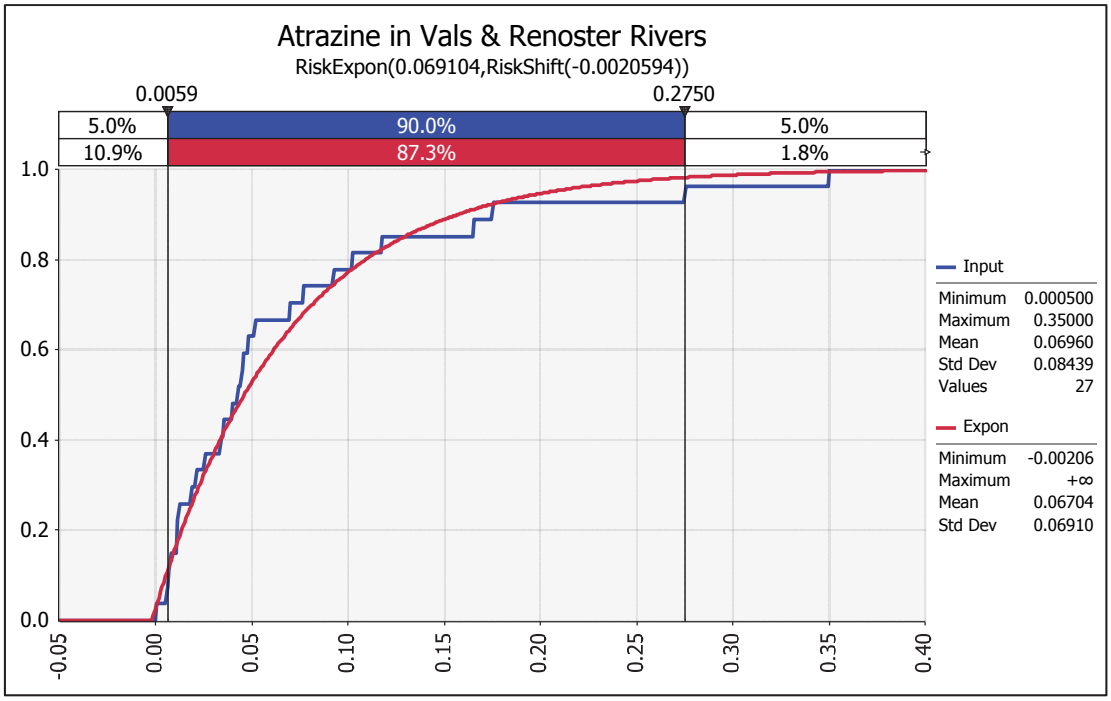

Figure 9.1: Distribution of atrazine in Vals-Renoster dataset

A summary of possible health effects associated with the pesticides detected in this study are presented in Table 9.4. All of the pesticides detected have possible endocrine disrupting activity and three are possible carcinogens (alachlor, atrazine, and simazine).

Table 9.4: Possible endocrine disrupting activity associated with pesticides detected in study site samples

Chemical	Carcinogenicity Data	Chemical description	Endocrine disrupting activity
Alachor	Probable human carcinogen at high doses	Herbicide	Binds competitively to oestrogen and progesterone receptors. Interacts with the pregnane X cellular receptor, interfering with the manufacture of enzymes responsible for steroid hormone metabolism. Decreased serum testosterone levels, increased 17b-estradiol levels and hepatic microsome GST activities were continuously induced (Yi *et al.*, 2007; Klotz *et al.*, 1996).
Atrazine	Probable carcinogen (US EPA)	Herbicide	Affects the serum androgen-oestrogen homeostasis and increases aromatase activity and decreases the transcription of steroidogenic genes significantly. Damages the adrenal glands and impairs steroid hormone metabolism (Jin *et al.*, 2013;Friedmann, 2002; Kucka *et al.* 2001;Albanito *et al.*,2008; Hayes *et al.*, 2003)
Carbofuran	Unlikely to be a human carcinogen (US EPA)	Carbamate Insecticide	Acute doses increase levels of progesterone, cortisol and oestradiol whilst decreasing testosterone levels (rats) (Goad *et al.*, 2004)
Diphenylamine	Unlikely to be a human carcinogen (US EPA)	Pesticide - plant growth regulator	Both impairment of reproduction as well as any specific embryo-/fetotoxic or teratogenic (SCHER, 2008). Very few activity studies. Anilines, in general, are capable only of very weak oestrogenic activity.
Imidacloprid	Group D, no evidence	Insecticide made to mimic nicotine.	Decreased ovarian weight together with significant patho-morphological changes in follicles, antral follicles and atretic follicles. Produced significant alterations in the levels of Luteinizing hormone, follicle stimulating

Chemical	Carcinogenicity Data	Chemical description	Endocrine disrupting activity
			hormone and progesterone (Kapoor *et al.*, 2011).Causes the induction of oxidative stress in testis (rats, Bal *et al.*, 2012).Causes endocrine disruption in humans by binding to and inhibiting the oestrogen receptor. Nuclear hormone receptor. The steroid hormones and their receptors are involved in the regulation of eukaryotic gene expression and affect cellular proliferation and differentiation in target tissues (Luft *et al.*, 2009).
Imazalil	Unlikely to be a human carcinogen (US EPA)	Imidazole fungicide	Inhibited aromatase activity in rainbow trout and chicken ovarian microsomes (5uM) (Monod *et al.*, 1993; Matsushita *et al.*, 2006).
Propiconazole	Group C, possible human carcinogen	Fungicide	Weak oestrogen inhibitor. Inhibits the enzyme aromatase, decreasing the production of oestrogens and increasing the available androgens; inhibit oestrogen biosynthesis just as effective or more as the androgen biosynthesis. Induced cell proliferation induced directly via the oestrogen receptor (Kjærstad *et al.*, 2010).
Simazine	Unlikely to be a human carcinogen (US EPA)	Herbicide	Induces aromatase activity, increasing oestrogen production. antiestrogenic/antiandrogenic activity in the yeast screen, and inhibition of ovulation *in vitro*, accompanied by decreased testosterone production (Orton *et al.*, 2009). Delays the onset of puberty in the female rat and decreases serum PRL. There is direct evidence for neuroendocrine disruption (US EPA, 2006).
Terbuthylazine	Group D, no evidence	An algicide, microbicide and microbiostat ; chloro-s-triazines	Human pregnane X receptor agonist. A weak inducer of aromatase activity. (Roberge *et al.*, 2004; Creusot *et al.*, 2010).
Thiabendazole	Carcinogenic at high doses	systemic benzimidazole fungicide	Confirmed to have oestrogen activity (US EPA, 2002b; Kojima *et al.*, 2005; Manabe *et al.*, 2006)

Studies with regards to the possible endocrine health effects of specific pesticides are slowly increasing, but are generally limited to impacts on first and second generations. Investigated concentration ranges also vary, and are generally in comparable regions for established no observed adverse effect level (NOAELS) for the individual pesticides. Very few studies attempt to evaluate multiple exposure effects stretching multiple generations.

9.3.2 Pesticide Health Effects

The next section provides a more detailed description of each of the pesticides detected in the study sites and potential health effects.

9.3.2.1 Alachlor

General

Alachlor is an herbicide used for weed control on corn, soybeans, sorghum, peanuts, and beans applied at pre-emergent, pre-plant, at plant and post plant situations. Alachlor is often applied with fertilizer and can dissolve into surface runoff after rainfall periods. Alachlor has a low affinity to adsorb to soils and is highly mobile. Monitoring studies (US EPA, 1998a) have shown significant groundwater contamination of Alachlor even when used at prescribed

dose concentrations. Alachlor and its associated degradation products are moderately persistent and dissipate primarily by aerobic soil metabolism processes with a half-life of 2-3 weeks.

Health effects

Alachlor has demonstrated hepatotoxicity, haemosiderosis, and uveal degeneration (US EPA, 1998a; WHO, 2003). Alachlor has not shown developmental or reproductive toxicity (rats and rabbits) and is not considered mutagenic. The US EPA has set the NOAEL and LOAEL at 1 mg/kg/day and 3 mg/kg/day respectively with an oral reference dose of 0.01 mg/kg/day.

Several metabolites of alachlor have, however, given positive genotoxic results (Brown *et al.*, 1988; Hill *et al.*, 1997; USEPA, 1998a). Animal (rat and mice) carcinogenicity studies have demonstrated tumors of the nasal turbinates, stomach, and thyroid only at either maximum tolerated doses or related to species-specific pathways (Heydens *et al.*, 1999; US EPA, 1998a; WHO, 2003). Alachlor is therefore considered to be a probable human carcinogen (B2 carcinogen) at high doses, but not likely at low doses and has not been assigned a slope risk factor.

ED Activity

Alachlor has been categorized as a category 1 EDC, and has shown endocrinal effect in at least one animal type (Hrouzková and Matisová, 2012; EC, 1999). In a crucian carp study (Yi *et al.*, 2007) alachlor bound competitively to oestrogen and progesterone receptors, interacted with the pregnane X cellular receptor and therefore interfered with the manufacture of enzymes responsible for steroid hormone metabolism. Alachlor inhibits binding to a progesterone receptor and competes with [3H]17 beta-oestradiol for binding to the oestrogen receptor.

Alachlor significantly decreased serum testosterone levels in the crucian carp compared to the control, but 17b-estradiol levels were significantly increased with large variation, and hepatic microsome GST activities were continuously induced (Yi *et al.*, 2007). Alachlor resulted in a marked inhibition to UDP-glucuronosyltransferase activity at all doses given to the crucian carp. The exposure of the fish to Alachlor resulted in changes in their serum sex steroid levels. Alachlor displayed weak oestrogenic activity in the *in vitro* assays (Klotz *et al.*, 1996).

Alachlor has been shown to cause increase thyroid weights at doses ~5-fold higher than the subchronic and chronic critical study LOAEL. Thyroid tumours were also observed in rats exposed to doses ~40-fold higher than the subchronic and chronic critical study LOAEL.

Alachlor was found to enhance steroid xenobiotic receptor (SXR)-mediated transactivation of human CYP3A2 and CYP3A4 gene (Zhou *et al.*, 2013). SXR, also known as NR1I2, regulates CYP3A expression in response to exogenous chemicals, after binding to SXRE (SXR response element). The study by Zhou *et al.* (2013) showed that alachlor also increased endogenous mRNA expression of CYP3A4 gene in HepG2 cells. The luciferase assay showed that alachlor could enhance SXR-mediated rat or human CYP3A gene transcription nearly evenly, and could also activate rat CYP7A1 gene transcription by cross-interaction of SXR and LXRE (LXRα response element) (Zhou *et al.*, 2013).

9.3.2.2 Atrazine

General

Atrazine is a selective triazine herbicide used to control broadleaf and grassy weeds in corn, sorghum, sugarcane, pineapple, Christmas trees, and other crops, and in conifer reforestation plantings. Atrazine is highly persistent in soil. Atrazine is moderately to highly mobile in soils with low clay or organic matter content. Because it does not adsorb strongly to soil particles and degrades slowly in water, it has a high potential for groundwater contamination despite its moderate solubility in water (Wauchope *et al.*, 1992).

Health effects

In mammalian studies, atrazine is rated as having low acute toxicity. Atrazine product formulations can be mild skin sensitizers and irritants. Chronic high dose toxicity observed in animals have demonstrated decreased body weight, myocardial muscle degeneration, liver toxicity, developmental ossification defects, impaired fertility, altered oestrus cycles, increased pituitary weight, delayed onset of puberty, and reduced levels of luteinizing hormone, prolactin, and testosterone (Eldridge *et al.*, 1994; Laws *et al.*, 2003; Rayner *et al.*, 2004; US EPA, 2003). Atrazine is not considered genotoxic. IARC considers atrazine not classifiable with respect to human carcinogenicity, and US EPA considers atrazine unlikely to be a human carcinogen. The US EPA has set a NOAEL of 3.5 mg/kg/day and a LOAEL of 25 mg/kg/day based on a 2 year dog study showing cardiac toxicity and moderate to severe dilution of the right atrium. The reference dose for the oral consumption of atrazine is 0.035 mg/kg/day.

ED activity

Of the EDCs evaluated in this study, Atrazine is by far the most extensively studied in the available literature. Like Alachlor, Atrazine has been classified as a category 1 EDC with evidence of ED activity in animal and human cell studies (Hrouzková and Matisová, 2012; EC, 1999). Atrazine acts as an endocrine disrupter in male rats by directly inhibiting Leydig cell testosterone production (Friedmann, 2002). Atrazine was also reported to have oestrogenic effects on tadpoles and adult frogs (Hayes *et al.*, 2003), converting adult males into hermaphrodites and retarding gonadal development. Atrazine has been reported to be an androgen inhibitor with a weak oestrogenic effect and also disrupts the hypothalamic control of lutenising hormone and prolactin levels. Other ED complications include damaging of the adrenal glands and impairment of steroid hormone metabolism (summarised by McKinlay *et al.*, 2008). Atrazine induces aromatase, the rate- limiting enzyme in the conversion of androgens to oestrogens, thereby increasing oestrogen production (Sanderson *et al.*, 2000). A 2008 study (Albanito *et al.*, 2008) also found that atrazine stimulates aromatase activity in human ovarian cancer cells.

Atrazine was shown to have a high impact on the menstrual cycles of woman working with close to/ with atrazine on a regular basis. Menstrual cycle characteristics have implications for women's fecundity and risk of chronic diseases such as osteoporosis and cancers associated with reproductive hormones. Compared with never mixing or applying pesticides, use of lindane or atrazine was associated with long cycles (OR = 2.7), missed periods (OR = 2.0), and intermenstrual bleeding (OR = 1.7). Use of these pesticides was also associated with decreased odds of irregular cycles (OR = 0.45). (Farr *et al.*, 2004). Exposure to atrazine

has also been shown to reduce semen quality in men living in agrarian areas (Swan *et al.*, 2003).

Atrazine, simazine and propazine induced aromatase activity in H295R cells concentration-dependently aromatase is the rate-limiting enzyme in the conversion of androgens to oestrogens (Sanderson *et al.*, 2000). Atrazine and cyanazine displaced [3H]17p-oestradiol from the a oestrogen receptor in the alligator (Vionier, 1996). Atrazine has been shown not to possess oestrogenic or antioestrogenic activity in the rodent or with the human ER (hER) using *in vitro* assays.

In a recent study (Jin *et al.*, 2013) the endocrine disruption effects of atrazine in peripubertal male mice was investigated. The mice experienced decreases the body weight and increases the relative testis weight. Atrazine affected the serum androgen-oestrogen homeostasis and increased aromatase activity and decreased the transcription of steroidogenic genes significantly, which may explain the induced endocrine disruption. Atrazine-induced changes in cAMP levels (in rats) were sufficient to stimulate prolactin release in pituitary cells and androgen production in Leydig cells (Kucka *et al.*, 2012), indicating that it acts as an endocrine disrupter both in cells that secrete by exocytosis of prestored hormones and in cells that secrete by de novo hormone synthesis.

9.3.2.3 Carbofuran

General

Carbofuran is a broad spectrum carbamate pesticide that kills insects, mites, and nematodes on contact or after ingestion. It is used against soil and foliar pests of field, fruit, vegetable, and forest crops (EXTOXNET, 1996). Carbofuran is soluble in water and is moderately persistent in soil. Carbofuran is mobile to very mobile in soil, it does not volatilize from water, nor does it adsorb to sediment or suspended particles (Howard, 1991).

Carbofuran is highly toxic to fish, bees and birds. "Millions" bird kill-offs have been a result of granular carbofuran for the past 40 years and were the main driving force behind the movement to cancel use of the pesticide. In 2008 the US EPA moved to revoke registration of carbofuran, effectively banning its use in agriculture (US EPA, 2009).

Health effects

The NOAEL for carbofuran was set at 0.5 mg/kg/day and was based on the following critical effects in a 1 year dog study; RBC and plasma cholinesterase inhibition, and testicular and uterine effects. The oral reference dose for carbofuran is set as 0.005 mg/kg/day (US EPA, 2009).

Carbofuran is highly toxic by inhalation and ingestion and moderately toxic by dermal absorption. Death may result at high doses from respiratory system failure associated with carbofuran exposure. Carbofuran causes cholinesterase inhibition in both humans and animals, affecting nervous system function. Consuming high doses over long periods of time caused damage to testes in dogs, but carbofuran did not have any reproductive effects on rats or mice (Baron, 1991). Available studies indicate carbofuran is unlikely to cause

reproductive effects in humans at expected exposure levels (Baron, 1991). Carbofuran is most likely non-mutagenic and does not pose a risk of cancer to humans.

ED activity

Carbofuran has been classified as a Category 2 EDC (Hrouzková and Matisová, 2012; EC 1999), and has been recorded to cause biological activity *in vitro* leading to possible endocrine disruption. Acute doses (1.5 mg/kg s.c) of carbofuran increased the levels of progesterone (1279%), cortisol (202%) and oestradiol (150%) whilst decreasing testosterone (88%) levels in adult rats (Goad *et al.*, 2004). In a study on the effects of carbofuran on rabbit semen (Yousefa *et al.*, 1995) results showed a decline in body weight, libido, ejaculate volume, sperm concentration, semen initial fructose and semen osmolality. This was accompanied with increases in the abnormal and dead sperm and semen methylene blue reduction time. These results suggest that an acute exposure to carbofuran may cause transient endocrine disruption, which may consequently lead to serious reproductive problems following repeated exposure.

Carbofuran is metabolized by cytochromes P450 into 3-hydroxycarbofuran. The oxidation of carbofuran by cytochromes P450 leads to activation of the detoxification pathways and elevated metabolism of testosterone (Usmani *et al.*, 2004). As hormone balance is altered, endocrine disruption follows.

In a New Jersey study (Barr *et al.*, 2010), the utero exposures to pesticides by measuring maternal and cord serum biomarkers of pregnant women and the birth outcomes of their neonates were investigated. Carbofuran was frequently detected in maternal and umbilical cord sera (4.36 ng/g), maternal blood carbofuran concentrations were significantly inversely correlated with cord blood concentrations. A nominally significant association was observed between cord serum concentrations of carbofuran and head circumference of the new-borns.

Carbofuran has been reported to increase serum concentrations of adrenal glucocorticoids in mice and rats, possibly by inhibiting metabolism by the liver (Cranmer *et al.*, 1978). Inhibition of the ability of the liver to metabolize corticosteroids by carbofuran may indicate a general inhibition of liver metabolic function, which could explain the increased thyroxine serum concentrations seen in ewes treated with carbofuran.

9.3.2.4 Diphenylamine

General

Diphenylamine (DPA) is a plant growth regulator used post-harvest on apples to control storage scald. It is moderately soluble in water (35-45 mg/L) and is stable towards hydrolysis at pH values of 5, 7, and 9 (summarized in Drzyzga, 2003). Many studies have shown that the toxic effects of DPA are generally more from the impurities of compounds such as nitrosamine contained in the commercially available packages of DPA (US EPA, 1998b; Crocker *et al.*, 1972). It is also often used in a DNA fragmentation method (Burton, 1956) in human health studies. DPA is readily biotransformed to hydroxylated metabolites and their conjugates and excreted; consequently no potential for bioaccumulation is expected.

Health effects

In reaching the determination of safety for infants and children, the US EPA found that the toxicity data base for diphenylamine is complete, based on current requirements, and the effects observed in pre- and post-natal studies do not indicate an increased sensitivity of infants and children to diphenylamine (US EPA , 1998b).

Eczema formation, hypertension, tachycardia, and bladder diseases have been observed when humans had been administered alcoholic DPA solutions (Bingham *et al*., 2001). DPA treatment in short-time studies and during long-term expositions in animal experiments with dogs, rats, and mice showed an increase of organ weights, methemoglobinaemia as well as liver, spleen, and kidney damaging effects (e.g. coagulations, necroses and lesions) (US EPA, 1998b). Negative results from an *in vivo* micronucleus test indicate that no mutagenic effects are expressed *in vivo*. Diphenylamine is non-genotoxic and is classified as "not likely" to be carcinogenic to humans (SCHER, 2008).

Regarding reproductive and developmental effects, data from laboratory animals are limited to studies with the oral route. Both impairment of reproduction as well as any specific embryo-/foetotoxic or teratogenic potential capability are unlikely to occur in the absence of parental toxicity (reduced body weight, decrease in food consumption and pathological findings in spleen, kidney and liver). For risk characterisation purposes, DPA NOAEL/fertility of 131 mg/kg bw/d, and NOAEL/developmental toxicity of 46 mg/kg bw/d have been established (SCHER, 2008).

ED activity

There is currently very little information provided in the literature on the possible ED activity of DPA. The compound is however listed by the US EPA as a compound to be investigated for possible classification as an EDC.

9.3.2.5 Imidacloprid

General

Imidacloprid is a systemic, chloro-nicotinyl insecticide with soil, seed and foliar uses for the control of sucking insects. The chemical works by interfering with the transmission of stimuli in the insect nervous system. The half-life of imidacloprid in soil is 48-190 days. Imidacloprid breaks down rapidly in water in the presence of light, has a relatively high solubility and has moderate binding affinity to organic materials in soils.

Based on its high water solubility (0.5-0.6 g/L) and persistence, both the US Environmental Protection Agency and the Pest Management Regulatory Agency in Canada consider imidacloprid to have a high potential to run off into surface water and to leach into ground water and thus warn not to apply in areas where soils are permeable, particularly where the water table is shallow

Health effects

The oral dose of technical grade imidacloprid that resulted in mortality to half of the test animals (LD50) is 450 mg/kg body weight in rats (Meister, 1994), and 131 mg/kg in mice. Consumption of imidacloprid results in decreased body weight and skeletal abnormalities and changes in chromosomes in human lymphocytes. Imidacloprid also tested positive for causing genotoxicity in Chinese hamster ovary cells. Imidacloprid is considered non-carcinogenic (Gervias *et al.*, 2010).

It is not listed for reproductive or developmental toxicity. A developmental neurotoxicity screening study in rats (Sheets, 2001) suggests that imidacloprid may cause neurotoxicity in offspring born to imidacloprid-exposed mothers at doses which do not cause maternal toxicity. Study conducted on rats suggests that the neonicotinoids may adversely affect human health, especially the developing brain (Gervias *et al.*, 2010).

ED Activity

Imidacloprid is included in the draft list of initial chemicals for screening under the US EPA Endocrine Disruptor Screening Program (EDSP) (US EPA, 2007). The list of chemicals was generated based on exposure potential, not based on whether the pesticide is a known or likely potential endocrine disruptor.

No pathological findings involving adrenal tissues were reported in the comprehensive acute, subchronic and chronic exposure studies conducted on rats, mice, and dogs with imidacloprid and imidacloprid formulations. However, feeding of technical grade Imidacloprid caused degenerative changes in the thyroid in dogs (follicular atrophy) (5000 ppm); in rats (mineralization of colloid follicles) (900 ppm) and in rats (1800 ppm) (summarised in USDA, 2005).

Imidacloprid was evaluated for its effect on ovarian morphology, hormones and antioxidant enzymes in female rats after 90 days oral exposure (Kapoor *et al.*, 2011). Decreased ovarian weight together with significant patho-morphological changes in follicles, antral follicles and atretic follicles were observed at 20 mg/kg/day. This dose has produced significant alterations in the levels of Luteinizing hormone, follicle stimulating hormone and progesterone. Similarly significant changes in superoxide dismutase, catalase, glutathione peroxidase, glutathione, and lipid peroxidation were also observed.

Bal *et al.,* (2012) observed a relationship between the dose of imidacloprid and the degree of sperm deterioration in rats. Exposure to sublethal dosages of imidacloprid affects the reproductive organ of male rats by decreasing the mass of accessory sex organs, testosterone level, sperm concentration, by increasing the rate of abnormal sperm morphology, by changing the lipid composition of testicular tissue, by fragmenting seminal DNA and by increasing apoptosis of spermatogenic cells. The adverse effect of imidacloprid on the reproduction of male rats appeared to be due to the induction of oxidative stress in testis. Exposure to a 90 study and 8 mg/kg dose of imidacloprid caused 75% of germ cells to die by spontaneous apoptosis, leading to abnormal spermatogenesis. The increased apoptotic index in the testicular tissue correlated with decreased sperm count and increased sperm abnormalities.

9.3.2.6 Imazalil

General

Imazalil is a systemic imidazole fungicide used to control a wide range of fungi on fruit, vegetables, and ornamentals. Imazalil is also used as a seed dressing and for postharvest treatment of citrus, banana, and other fruit to control storage decay. It is applied as a broad spectrum antimycotic in cattle, horses and dogs (EMEA, 1999).

Imazalil is highly persistent in the soil environment, with a reported field half-life of between 120 and 190 days. It is soluble in water, but strongly bound to soils, and thus unlikely to pose a risk to groundwater (Wauchope *et al.*, 1992).

Health effects

Imazalil is moderately toxic by ingestion, with a reported oral LD50 of 227 to 343 mg/kg in rats. Mice studies suggest that imazalil is unlikely to be carcinogenic to humans. In 2 year dose experiments with mouse, dog and rat, the liver was identified as the main target organ for toxicity. The effects included centri-lobular swelling, fatty surcharge and numerous vacuoles (20 mg/kg bw) (EMEA, 1999). There are also no direct embryotoxic effects of imazalil and also no signs of mutagenic activity (EMEA, 1999). At a dose range of 5-20 mg/kg bw imazalil was fetotoxic in rats, mice, and rabbits.

ED activity

Imazalil is classified as a Category 3 EDC by the EC commission saying that there is not enough evidence to confirm/ or disconfirm its potential endocrinal activity (Hrouzková and Matisová, 2012; EC, 1999).

Imazalil was tested for its ability to activate the oestrogen receptor *in vitro* in a proliferation assay with MCF7 cells and a screening test for yeast oestrogen. Imazalil exhibited an almost negligible stimulation of cell proliferation of MCF-7, but found that it gave rise to a weakly positive oestrogenic response in yeast cells transfected with ER (Vinggaard *et al.*, 1999). Although, Imazalil showed no binding activity with an oestrogen acceptor, it inhibited the binding of testosterone to the androgen receptor at a concentration of approximately 36 µM (Okubo *et al.*, 2004).

11beta-Hydroxylation and 19-hydroxylation in the mitochondria of gerbils (*Meriones unguiculatus*) *in vitro* were suppressed in parallel by about 75% by imazalil at 1 µmol/L (Drummond *et al.*, 1988). Imazalil inhibited CYP19 aromatase activity, which catalyzed the conversion of androgens to oestrogens and thereby affected hormone levels and had influence on the homeostatic balance between the male and female sex hormones (Vinggaard *et al.*, 2000). The *in vitro* potency is estimated at was 3.23 µmol/L (Vinggaard *et al.*, 2000).

Aromatase can be inhibited competitively and reversibly by azole compounds, as seen with sterol 14α-demethylase. The IC50 value of imazalil on microsomal aromatase from human placenta was 0.34 µM. Imazalil inhibits *in vitro* aromatase activity in the chick ovary. The results indicated that in ovo exposure to imazalil inhibits sexual differentiation of the ovary by inhibiting aromatase activity (Matsushita *et al.*, 2006). Imazalil exposure amounting to 2 mg/egg inhibits hatch-ability.

9.3.2.7 Propiconazole

General

Propiconazole is registered for as a grass, produce and antimicrobial pesticide. The triazole fungicides share common metabolites, the free triazole compounds 1,2,4-triazole, triazole alanine, and triazole acetic acid. Propiconazole is a mixture of four stereoisomers, making detection, and toxicity studies somewhat complicated (Toribio *et al.*, 2004).

Propiconazole appears to be persistent and moderately mobile to relatively immobile, and therefore stable, in most soil and aqueous environments. Therefore, propiconazole may reach groundwater in soils with low organic content. More importantly, propiconazole may contaminate surface water through off-site runoff and spray drift.

Health effects

Based on a 1 year dog study, the NOEL for the consumption of propiconazole is 1.25 mg/kg/day. This was used to determine the reference dose for humans, 0.013 mg/kg/day.

An ADI of 0.0.04 mg/kg bw was allocated based on a NOEL of 4 mg/kg bw slight effects on the liver and haematology parameters at 20 mg/kg bw in a 2-year rat study. In a rat reproduction study, reduced testis and epididymis weights in pups were observed at 21 mg/kg bw (US EPA, 1996).

Exposure to 0.25 mg/L propiconazole caused a significant incidence of developmental abnormalities and embryonic death of the crustacean *Daphnia magna*. Results indicated that propiconazole interferes with the later stages of daphnid embryonic development, and that this toxicity is manifested largely via maternal exposure to the fungicide (Kast-Hutcheson *et al.*, 2001).

In two-year feeding studies in mice, the NOEL was established at 100 ppm. Significant increases were noted in the incidence of spontaneous liver tumours (benign) observed in male mice at the highest feeding level only. Over a two-year period, rats and their offspring were fed a diet containing propiconazole at concentrations up to 2,500 ppm. Although the high dose resulted in diminished body weight, and increases in relative liver weight of adults and pups, no reproductive, foetal, or embryonic parameters were affected. In a reproductive toxicity test with rats, doses as low as 30 mg/kg caused skeletal deformations in new-born pups. Reduced litter size and pup weight were observed at the 8mg/kg dose level (EC, 2003).

Developmental toxicity was observed in rats at 90 mg/kg/day; these effects occurred in the presence of maternal toxicity. In rabbits increased abortions were observed at the maternally toxic dose of 400 mg/kg/day.

ED activity

Propiconazole was classified as a Category 3 EDC by the EC commission saying that there is not enough evidence to confirm/ or disconfirm its potential endocrinal activity (Hrouzková and Matisová, 2012; EC, 1999).There has fortunately been numerous recent studies investigating the endocrine properties of propiconazole and it is now seen as a definitely EDC.

Propiconazole is a weak oestrogen and aromatase inhibitor. It inhibits the enzyme aromatase, decreasing the production of oestrogens and increasing the available androgens (Hurst and Sheahan, 2003; Trosken, 2004). In the H295R cell assay, the conazoles decreased the formation of estradiol and testosterone, and increased the concentration of progesterone, indicating inhibition of enzymes involved in the conversion of progesterone to testosterone. Propiconazole exhibits the least potency compared to similar structured conazoles. In the MCF-7 cell proliferation assay, the conazoles showed anti-oestrogenic effect, including aromatase inhibition, since they inhibited the response induced by both 17β-estradiol and testosterone. Triazoles are anti-androgenic in an androgen receptor reporter gene assay, with propiconazole presenting the lower potency (Kjærstad *et al.*, 2010).

Propiconazole has been shown to inhibit Cyp19 aromatase *in vitro* (Vinggaard *et al.*, 2000). Exposure to propiconazole induced expression of constitutive androstane receptor (CAR) and pregnane X receptor (PXR) stimulated pathways which are involved in xenobiotic metabolism and clearance in the liver (Goetz *et al.*, 2006). Since both CAR and PXR regulate the expression of CYP genes and the associated enzyme activity of oxidative metabolism of steroid hormones as well as xenobiotics, it may be that CAR and PXR are also components of a regulatory network that regulates circulating hormone levels.

A common mode of action for the reproductive toxicity of the triazole fungicides appears to be disruption of testosterone homeostasis. Elevated serum testosterone, increased testis weights and AGD, and hepatomegaly indicative of altered liver metabolism of steroids are the key events consistent with this common mode of action.

9.3.2.8 Simazine

General

Simazine is a selective triazine herbicide. It is used to control broad-leaved weeds and annual grasses in field, berry fruit, nuts, vegetable and ornamental crops, turfgrass, orchards, and vineyards. It has low water solubility, making it less mobile and limiting its leaching potential (Weed Science Society of America, 1994). Simazine is persistent and does not adsorb strongly to soil particles. In combination with a lengthy soil half-life, these factors suggest that simazine is likely to contaminate groundwater.

Health effects

Observed effects in test animals include tremors, damage to the testes, kidneys, liver, and thyroid, disturbances in sperm production, and gene mutations (US EPA, 1988). Long term exposure to simazine has the potential to cause tremors; damage to testes, kidneys, liver and thyroid; gene mutations. Simazine is likely either nonmutagenic or weakly mutagenic and does not appear to be teratogenic.

Although initial evaluations indicated that there may be a cancer risk to humans exposed to Simazine had been classified as “unlikely to be carcinogenic to humans” in 2006 (US EPA, 2006).

Simazine is slightly to practically nontoxic. In a sub-chronic developmental toxicity study, incomplete or absent bone formation or ossification was observed in foetal rats following

exposure of pregnant rats to simazine. After subchronic and chronic exposure to simazine, a variety of species were shown to exhibit neuroendocrine effects resulting in both reproductive and developmental consequences that are considered relevant to humans. Simazine is genotoxic and has been shown to have immunosuppressive (Kim *et al.*, 2002) and have endocrine disrupting activities (Orton *et al.*, 2009).

ED activity

There is direct evidence that simazine is associated with neuroendocrine disruption. Direct measurements of serum hormones such as certain steroid hormones and luteinizing hormone, as well as changes in oestrus cycling and histomorphic changes in hormone responsive tissues, indicate neuroendocrine disruption. For simazine, a neuroendocrine endpoint was identified for short-term exposure based on an adverse effect of delayed preputial separation observed in a pubertal study on male rats exposed to atrazine. Neuroendocrine effects such as this are the primary toxicological effects of regulatory concern for simazine. The NOAEL determined for short-term exposure is 6.25 mg/kg/day. The MOE level of concern includes an uncertainty factor of 100X which includes 10X each for Category 2 EDC (US EPA, 2006).

Simazine is an inducer of aromatase and may thereby act as an endocrine disrupter by increasing cellular oestrogen levels (Rakitsky *et al.*, 2000; Sanderson *et al.*, 2000). The observed induction of aromatase, the rate-limiting enzyme in the conversion of androgens to oestrogens, may be an underlying explanation for some of the reported hormonal disrupting and tumour promoting properties of these herbicides *in vivo* (Sanderson *et al.*, 2000). Induction of this rate-limiting enzyme *in vivo* would be expected to lead to increased local production of oestrogens with the potential to cause or contribute to oestrogen-mediated pathologies, such as the tumour promotion observed experimentally in atrazine-exposed rats (Sanderson *et al.*, 2000). Comparative studies between simazine and atrazine have suggested that structural similarity can serve as a general guide for predicting effects in *in vitro* test systems, but it is necessary to test individual compounds to determine specific potencies and mechanisms of action. Simazine can also act as a xenoestrogens through oestrogen receptor binding and/ or anti-androgenic mechanisms (Kojima *et al.*, 2004; Laville *et al.*, 2006).

Simazine-exposed male offspring (rat) showed decreased body, testicular, and epididymis weight, increased testicular apoptosis, and decreased sperm concentrations. Maternal exposure interferes with the pleotropic relaxin-NO signalling pathway, impairing normal development and reproductive activity of male offspring (Park and Bae, 2012). Studies have demonstrates that simazine delays the onset of puberty in the female rat and decreases serum prolactin similar to other chlorotriazines (Zorrilla *et al*., 2010).

Significant association between prostate cancer risk and exposure to simazine (1.89-fold excess risk for those highly exposed compared with those not exposed) was observed among 1516 prostate cancer cases and 4994 age-matched controls in a population-based case-control study in British Columbia, Canada (Band *et al.*, 2011). In situ oestrogen biosynthesis in the prostate takes place through aromatization of androgens by the enzyme aromatase resulting in prostate stromal and epithelial cell proliferation, and may also lead to oestrogen metabolites that form DNA adducts potentially leading to mutations and tumor initiation (Carruba, 2007; Band *et al.*, 2011).

Since simazine appears to be genotoxic, it has been proposed that chlorotriazine (atrazine and simazine) administration promotes the development of mammary gland tumors by inducing a premature reproductive senescence and thus creating an endocrine milieu conducive to tumor growth (female rats, Stevens *et al.*, 1994). Thus, chlorotriazines do not possess any intrinsic oestrogenic activity. Their capacity to induce mammary tumors is attributed to disruption of the estrus cycle, with episodes of persistent oestrus and inhibition of the surge of pituitary luteinizing hormone needed to stimulate ovulation, which may result in prolonged periods of oestrus. The mechanisms of tumorigenic action and oestrus cycle disruption are most likely of a threshold nature (Rakitsky *et al.*, 2000).

9.3.2.9 Terbuthylazine

General

Terbuthylazine is an algicide, microbicide and microbiostat used to control slime-forming algae, fungi, and bacteria. In the early 1990 the use of atrazine was banned in many countries and was replaced by terbuthylazine. In a few years, terbuthylazine became a chemical of concern, together with its main metabolite desethylterbuthylazine (DET), because of alerting detections in surface and groundwater resources. Terbuthylazine is stable in neutral, weakly acid, and weakly alkaline media. Degradation of terbuthylazine in aquatic environment depends on the presence of sediments and biological activity

Health effects

Terbuthylazine generally is of relatively low acute toxicity. In chronic toxicity and carcinogenicity studies using mice and rats, decreases in body weight gain and food consumption were observed. A study using rats caused an increased incidence of testicular tumors in males and mammary gland carcinomas in females, but only at a dose at which excessive systemic toxicity also was observed. Terbuthylazine is classified as a Group D carcinogen, one for which there is inadequate evidence to determine carcinogenicity in humans.

In a study using rats, maternal toxicity was observed as reduced body weight gain and food intake, and developmental toxicity was observed in the litter with chronic exposure to terbuthylazine. Available studies indicate that terbuthylazine is not mutagenic (US EPA 1995).

In a 2-generation dietary study in rats, at 0, 6, 60 or 300 mg/kg diet, decreased body-weight gain and food/water consumption, and slightly higher infertile pairings and pup mortality, and retarded pup growth were noted at the high dose (EFSA, 2011). Male rats were more sensitive than females to the effects of terbuthylazine. In an oral study on pregnant rats given terbuthylazine effects were seen on foetal development (delayed/ absent ossification of phalanges) at 30 mg/kg bw/d (EFSA, 2011).

Results suggest that terbuthylazine induced oxidative stress in embryo-larval stages of common carp. Increased activities of biotransformation enzymes or antioxidant defence enzymes were markers of free radical attack. There was low lipid peroxidation in early developed fish after triazine exposure. The exposure of common carp to terbuthylazine

showed significant differences in weight and growth parameters at concentrations of 520 and 820µg/L. The change in growth and weight parameters were associated with a delay in development. The same effects were seen with zebrafish (Plhalova *et al.*, 2012).

ED activity

In vitro, terbuthylazine showed weak to moderate human PXR activation. As a nuclear receptor, PXR acts as a transcription factor and, following ligand binding, functions as a heterodimer with retinoid X-receptor (RXR) in a non-permissive way, and is thus involved in the metabolism of endogenous and exogenous compounds. The EC50 of terbuthylazine was identified to be 3.34 E-05 (mol/L), while the maximal luciferase induction of 10 µM terbuthylazine was 32±1% (Creutos et al., 2010). The ED activity of concern is therefore Human pregnane X receptor (hPXR) agonist. PXR controls the transcription of phase I cytochrome P450(CYP) genes, phase II conjugating enzymes, as well as phase III transporter genes.

Fluorescence polarization indicated that the binding equilibria between oestrogen receptor-α or oestrogen receptor-β, and oestradiol were not affected by terbuthylazine and ATR metabolites (Roberge *et al*., 2004). Terbuthylazine, along with other atrazine degradation products were also found to have a very weak ability(compared to atrazine) to inhibit phosphodiesterase (PDE), the enzyme responsible for hydrolyzing the second messenger cAMP to 5′-AMP (Roberge *et al.*, 2004). A reduction in PDE activity would result in increased levels of cAMP. Elevated levels of cAMP could possibly lead to aromatase induction in the H295R cell line (Sanderson *et al*., 2000).From structural similarities it therefore likely that a weak EDC activity for terbuthylazine may be due to the increase in aromatase, increasing oestrogen production.

9.3.2.10 Thiabendazole

General

Thiabendazole is a systemic benzimidazole fungicide used to control fruit and vegetable diseases. In livestock and humans, it is applied to treat several helminth species such as roundworms. Thiabendazole is also used medicinally as a chelating agent to bind metals.

Thiabendazole's affinity for binding to soil particles increases with increasing soil acidity. Due to its binding and slight solubility in water, it is not expected to leach readily from soil.

Health effects

Thiabendazole generally has been shown to have low acute dermal toxicity. It is neither irritating to the eyes or skin nor is a dermal sensitizer. Toxicity categories, which range from 1 (most toxic) to 4 (least toxic), were mostly 4 for thiabendazole. The thyroid and liver are the primary target organs of thiabendazole.

The principal target organs of thiabendazole in short term, repetitive dosing studies were histopathological changes in the liver, the kidney, and the thyroid. The effects of chronic exposure to thiabendazole were thyroid toxicity, hepato/biliary toxicity, anaemia and atrial thrombosis. These genotoxic effects involved spindle disruption, which is consistent with

thiabendazole's known ability to disrupt tubulin assembly. Thiabendazole caused major malformations of the skeletal system in mice, rabbits, and rats. When pregnant rats were exposed to thiabendazole in the diet, newborns presented increased malformations of the skeletal system, including cleft palates and the absence of the os hyoideus (US EPA, 2002b).

The US EPA has classified thiabendazole as "likely to be carcinogenic" at doses high enough to cause disturbance of the thyroid hormone balance. It is not likely to be carcinogenic at doses lower than those which could cause a disturbance of this hormonal balance. The Agency is using the MOE approach (of 13, 000) for the human cancer risk assessment. A MOE of 13,000 means that potential exposures to humans is 13,000 times less than the exposure to rats at which no adverse effects were observed.

ED Activity

Thiabendazole has adverse pre-natal effects and causes disruption of endocrine levels associated with body metabolism (US EPA, 2002b).The compound was associated with a wide spectrum of developmental toxicity in three species of laboratory animals (mouse, rat and rabbit), ranging from the induction of major malformations to foetal resorption (abortion). The lowest NOEL for this type of developmental toxicity was 24 mg/kg-day based on foetal resorption and hydrocephaly in rabbits. Thiabendazole has not been shown to adversely affect reproduction. There is evidence to suggest that it causes an elevation of thyroid stimulating hormone (TSH), resulting in hypertrophy of the thyroid gland (US EPA, 2002b).

The oestrogenic activity of thiabendazole was found using the E-CALUX assay which utilizes human ovarian carcinoma cells (BG1) stably transfected with an oestrogen-responsive luciferase reporter gene plasmid. The metabolites of thiabendazole, however, exhibited less oestrogenic activity than the original compounds (Kojima *et al*., 2005). The oestrogenic activity of thiadendazole was confirmed with the cell proliferative activity of MtT/Se cells, which respond well to oestrogen. Therefore, we evaluated the oestrogenic activity of a mixture of two pesticides. A pesticide mixture of thiabendazole/orthophenylphenol were increased the REC10 levels up to 10-fold, therefore having significantly higher oestrogenic effect in comparison to the pesticides when tested individually (Manabe *et al*., 2006).

9.3.3 Risk Characterisation

Hazard quotients and the risk of developing cancer resulting from exposure to the pesticides detected in the three study sites are summarised in Figure 9.2 to Figure 9.4 and are presented in more detail in Table 9.5 to Table 9.16. Each of the tables presents hazard quotients for anticipated toxic effects and cancer risks for the different sites and different scenarios used in the health risk assessment. The exposure parameters and calculations used in the calculations are presented in appendix 3. Different exposures were considered to provide an indication of possible adverse health effects through all different exposure routes and to identify a major contributor to overall risk. This included exposure if the water was used for direct consumption, if fish that had lived in the water were ingested, if the water was used for livestock and produce production or if the water was used for swimming or bathing. Air was also considered as an exposure route taking into account inhalation of pesticide via spray-drift at different distances from the point of application, ranging from the

point of application to 400 m from the point of application. Deposition of pesticides at varying distances was also used to calculate the concentration of pesticides a person might ingest via incidental ingestion of soil. These results are provided in Table 9.17.

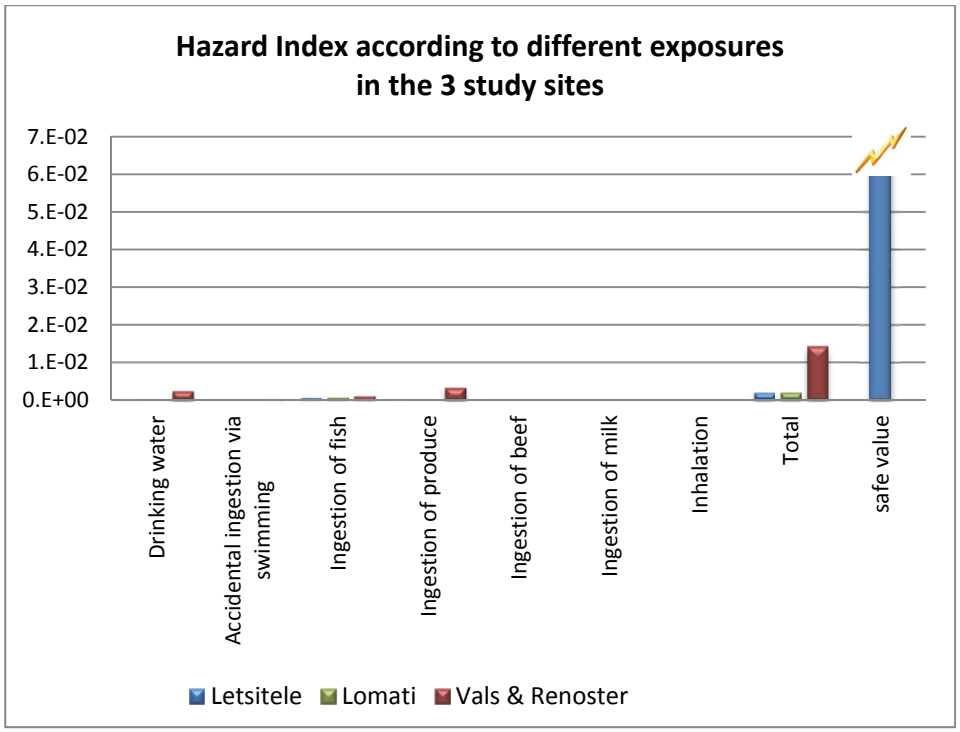

Figure 9.2: Hazard Quotients resulting from exposure to pesticides in the three study areas

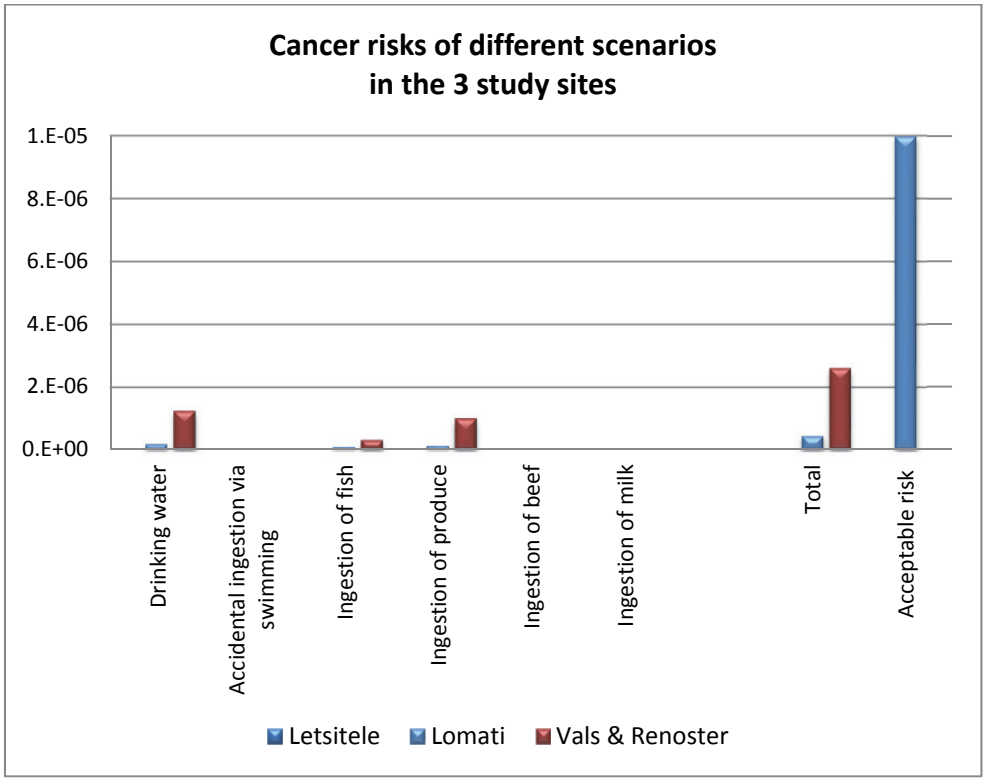

Figure 9.3: Cancer risks resulting from exposure to pesticides in the three study areas

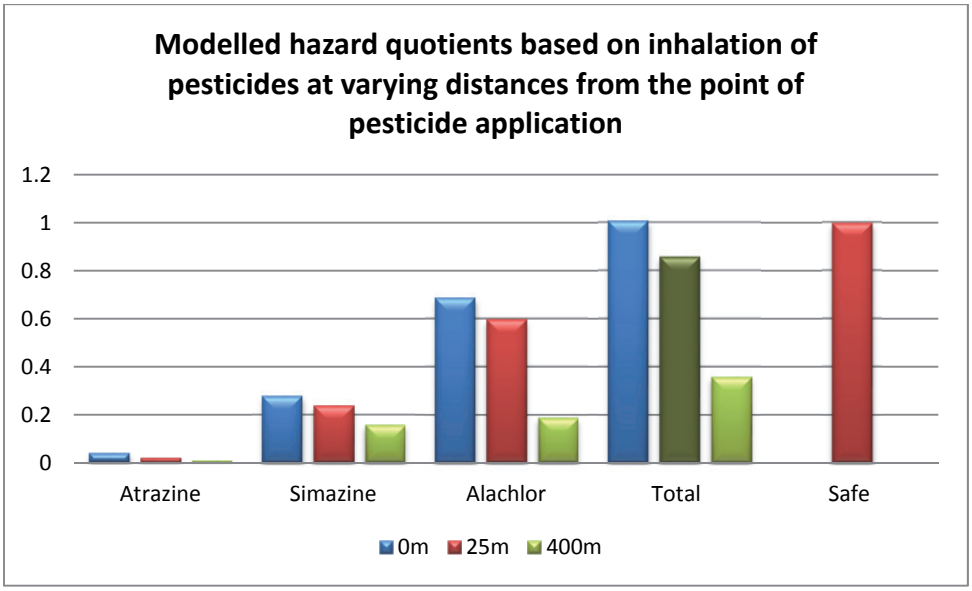

Figure 9.4: Modelled hazard quotients based on inhalation of pesticides at varying distances from the point of pesticide application

Uncertainty analyses using Monte Carlo simulations for each of the study sites are illustrated via probability distributions for total hazard indices in Figure 9.5. to Figure 9.7.

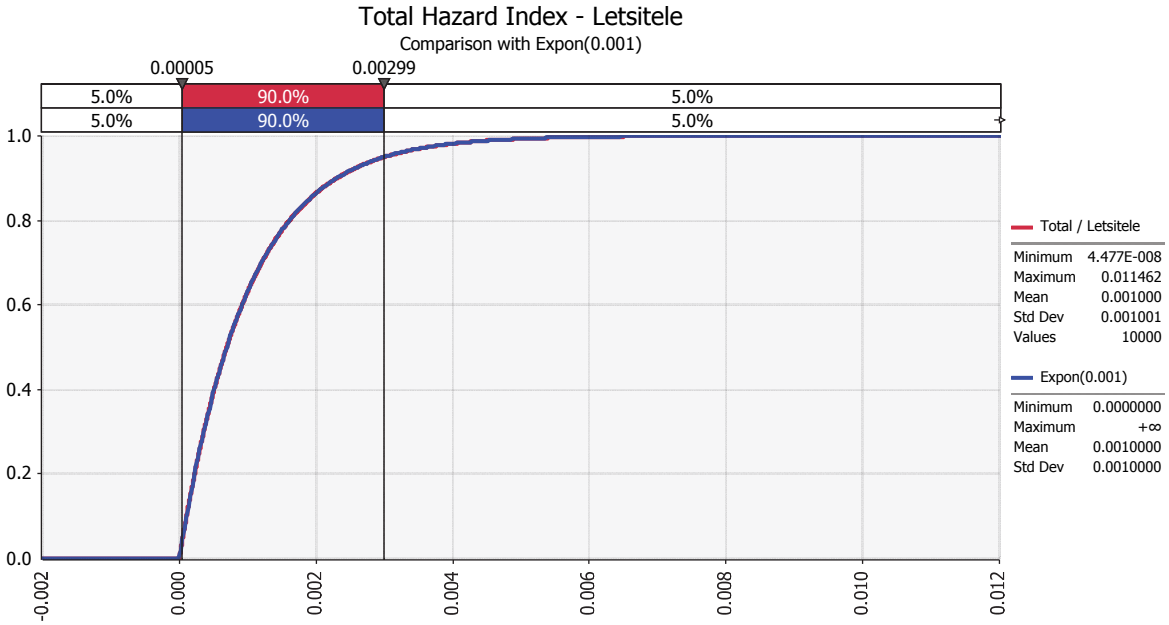

Figure 9.5: Monte Carlo simulation probability distribution for the Letsitele catchment.

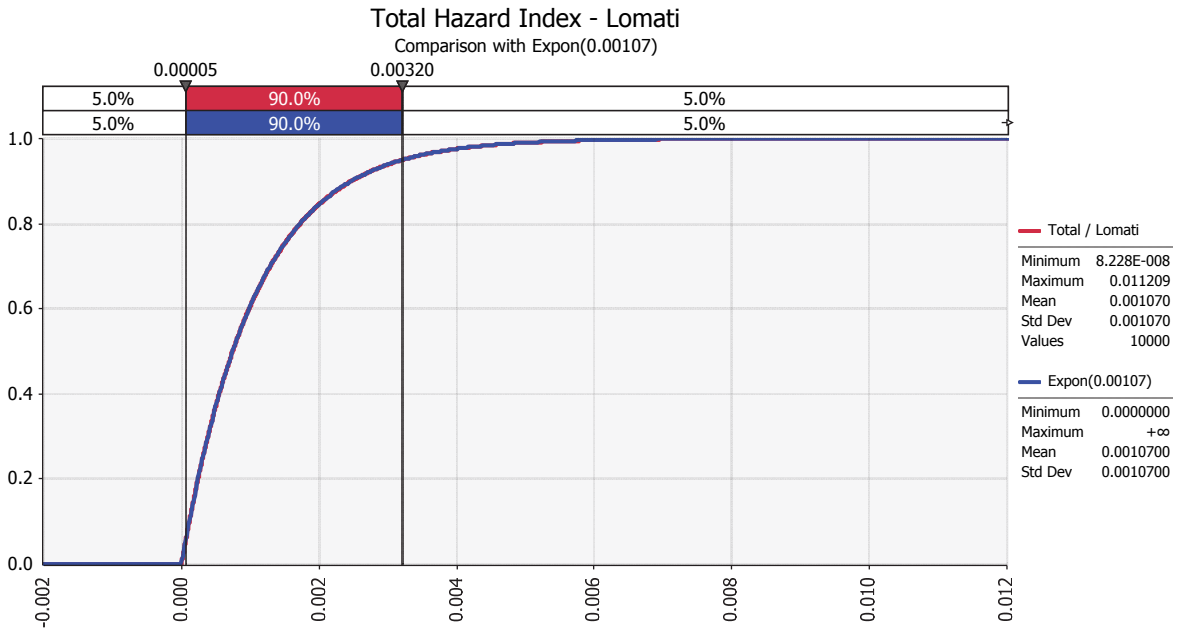

Figure 9.6: Monte Carlo simulation probability distribution for Lomati catchment

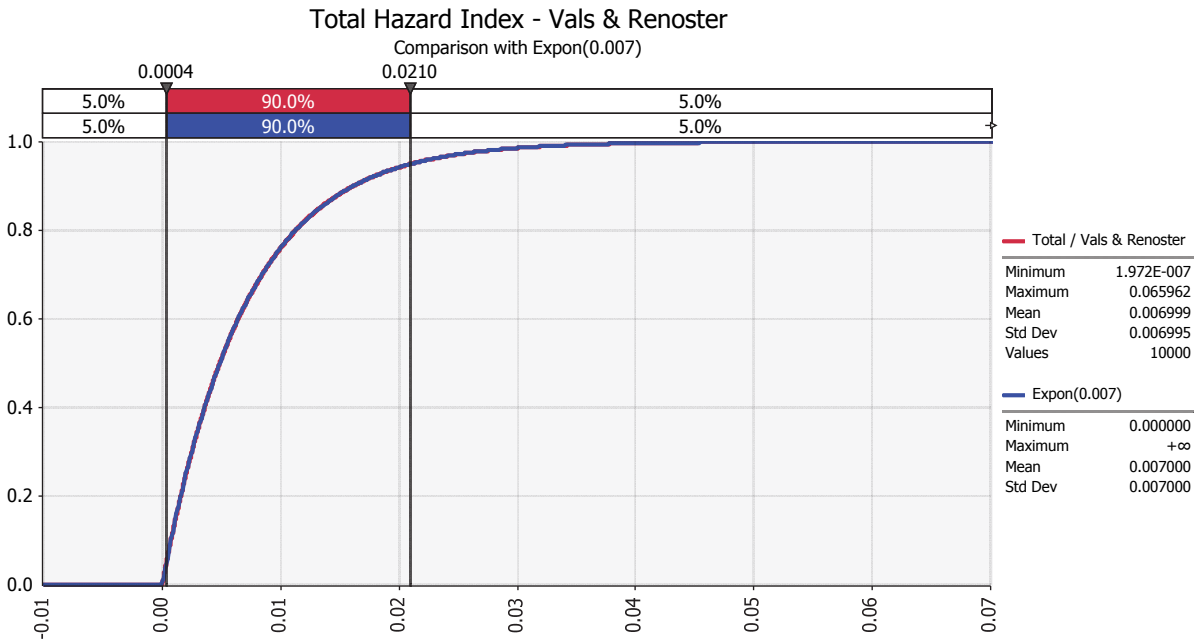

Figure 9.7: Monte Carlo simulation probability distribution for Vals & Renoster catchments

Based on ingestion of water and food, both hazard quotients and risks of developing cancer were many orders of magnitude lower than what is considered safe or acceptable for each of the three study sites. Risks of developing cancer were predicted at very low levels as a result of exposure to atrazine in Lomati. The Vals-Renoster samples contained simazine, atrazine and alachlor which were responsible for predicted cancer effects at levels lower than the excess cancer risk considered as acceptable. Hazard quotients were many orders of magnitudes lower than '1' which is considered safe for a lifetime exposure. The Letsitele

hazard quotients were three orders of magnitude lower than the allowable exposure (or 1/1000th of the safe dose) and no cancer risks were predicted. The Lomati hazard quotients were close to 1/100th of that considered safe with cancer risks 1000 times less than an acceptable risk. The Vals-Renoster hazard quotients were the highest with hazard quotients close to 50 times lower than the safe level and cancer risks slightly lower than the 1 in 100 000 acceptable risk.

Based on inhalation of pesticides, modelled air concentrations illustrated that of the three pesticides included in the assessment, alachor presented a higher risk than atrazine and simazine, but were within Occupational Health safe levels with hazard quotients ranging from 1% to 20% of the safe threshold values. These values are not used to protect public health however as they do not typically take into account sensitive individuals.

Overall the predicted adverse health effects based on exposure to the pesticides detected in water were considered to be very low. Exposure to pesticides via air presents a greater risk with possible adverse health effects.

9.3.4 Letsitele Risk Assessment

Table 9.5: Hazard Quotients and cancer risks resulting from exposure to water used for domestic purpose

Letsitele Resident RISK for water max values													
Chemical	**Concentration (µg/L)**	**Child Ingestion HQ**	**Child Dermal HQ**	**Child Total HI**	**Adult Ingestion HQ**	**Adult Dermal HQ**	**Adult Total HI**	**Adjusted Ingestion HQ**	**Adjusted Dermal HQ**	**Adjusted Total HI**	**Ingestion Risk**	**Dermal Risk**	**Total Risk**
Diphenylamine	0.0272	8.69E-05	5.71E-05	1.44E-04	3.73E-05	2.54E-05	6.27E-05	4.72E-05	3.11E-05	7.83E-05	-	-	-
Imazalil	0.0621	3.05E-04	1.28E-04	4.34E-04	1.31E-04	5.70E-05	1.88E-04	1.66E-04	6.97E-05	2.35E-04	-	-	-
Imidacloprid	0.0202	2.15E-05	6.75E-08	2.16E-05	9.22E-06	3.01E-08	9.25E-06	1.17E-05	3.67E-08	1.17E-05	-	-	-
Propiconazole	0.0043	2.11E-05	5.71E-06	2.69E-05	9.06E-06	2.54E-06	1.16E-05	1.15E-05	3.10E-06	1.46E-05	-	-	-
Thiabendazole	0.036	2.30E-05	-	2.30E-05	9.86E-06	-	9.86E-06	1.25E-05	-	1.25E-05	-	-	-
*Total Risk/HI	-	*4.58E-04*	*1.91E-04*	*6.49E-04*	*1.96E-04*	*8.50E-05*	*2.81E-04*	*2.49E-04*	*1.04E-04*	*3.53E-04*	-	-	-

Table 9.6: Hazard Quotients and cancer risks resulting from exposure to water used for swimming

Letsitele Recreation RISK for water max value													
Chemical	**Concentration (µg/L)**	**Child Ingestion HQ**	**Child Dermal HQ**	**Child Total HI**	**Adult Ingestion HQ**	**Adult Dermal HQ**	**Adult Total HI**	**Adjusted Ingestion HQ**	**Adjusted Dermal HQ**	**Adjusted Total HI**	**Ingestion Risk**	**Dermal Risk**	**Total Risk**
Diphenylamine	0.0272	5.59E-07	7.34E-06	7.90E-06	1.20E-07	4.29E-06	4.41E-06	2.07E-07	4.90E-06	5.11E-06	-	-	-
Imazalil	0.0621	1.96E-06	1.65E-05	1.84E-05	4.21E-07	9.63E-06	1.00E-05	7.29E-07	1.10E-05	1.17E-05	-	-	-
Imidacloprid	0.0202	1.38E-07	8.68E-09	1.47E-07	2.96E-08	5.07E-09	3.47E-08	5.13E-08	5.80E-09	5.71E-08	-	-	-
Propiconazole	0.0043	1.36E-07	7.34E-07	8.70E-07	2.91E-08	4.29E-07	4.58E-07	5.04E-08	4.90E-07	5.40E-07	-	-	-
Thiabendazole	0.036	1.48E-07	-	1.48E-07	3.17E-08	-	3.17E-08	5.49E-08	-	5.49E-08	-	-	-
*Total Risk/HI	-	*2.94E-06*	*2.46E-05*	*2.75E-05*	*6.31E-07*	*1.44E-05*	*1.50E-05*	*1.09E-06*	*1.64E-05*	*1.75E-05*			

Table 9.7: Hazard Quotients and cancer risks resulting from exposure to water used for the production of livestock and produce

Letsitele Farmer RISK for Contaminated Soil Max values							
Chemical	**Soil Concentration (mg/kg)**	**Produce Ingestion HQ**	**Beef Ingestion HQ**	**Milk Ingestion HQ**	**Produce Ingestion Risk**	**Beef Ingestion Risk**	**Milk Ingestion Risk**
Diphenylamine	0.001	0.0000214	1.97E-08	1.81E-07	-	-	-
Imazalil	0	0	0	0	-	-	-
Imidacloprid	0.000006	4.19E-08	-	-	-	-	-
Propiconazole	0	0	0	0	-	-	-
Thiabendazole	0.0003	0.00000126	-	-	-	-	-
*Total Risk/HI	-	*0.0000227*	*1.97E-08*	*1.81E-07*	-	-	-

Table 9.8: Hazard quotients and cancer risks resulting from ingestion of fish caught in the rivers

Letsitele Resident RISK for Fish Max values			
Chemical	**Surface Water Concentration (mg/L)**	**Ingestion HQ**	**Ingestion Risk**
Diphenylamine	0.0000272	1.22E-04	-
Imazalil	0.0000621	5.44E-04	-
Imidacloprid	0.0000202	-	-
Propiconazole	4.30E-06	3.23E-05	-
Thiabendazole	0.036	-	-
*Total Risk/HI	-	*6.98E-04*	-

9.3.5 Lomati Risk Assessment

Table 9.9: Hazard Quotients and cancer risks resulting from exposure to water used for domestic purposes

Lomati Resident RISK for water Max value													
Chemical	**Concentration (µg/L)**	**Child Ingestion HQ**	**Child Dermal HQ**	**Child Total HI**	**Adult Ingestion HQ**	**Adult Dermal HQ**	**Adult Total HI**	**Adjusted Ingestion HQ**	**Adjusted Dermal HQ**	**Adjusted Total HI**	**Ingestion Risk**	**Dermal Risk**	**Total Risk**
Atrazine	0.192	4.09E-03	5.09E-04	4.60E-03	1.75E-03	2.27E-04	1.98E-03	2.22E-03	2.77E-04	2.50E-03	6.57E-07	8.20E-08	7.39E-07
Carbofuran	0.0561	7.17E-04	5.53E-05	7.73E-04	3.07E-04	2.46E-05	3.32E-04	3.89E-04	3.01E-05	4.19E-04	-	-	-
Imidacloprid	0.0647	6.89E-05	2.16E-07	6.92E-05	2.95E-05	9.63E-08	2.96E-05	3.74E-05	1.18E-07	3.75E-05	-	-	-
Terbutylazine	0.02	3.20E-04	1.71E-04	4.91E-04	1.37E-04	7.61E-05	2.13E-04	1.74E-04	9.30E-05	2.67E-04	-	-	-
Thiabendazole	0.0069	4.41E-06	-	4.41E-06	1.89E-06	-	1.89E-06	2.39E-06	-	2.39E-06	-	-	-
*Total Risk/HI	-	*5.20E-03*	*7.36E-04*	*5.94E-03*	*2.23E-03*	*3.28E-04*	*2.56E-03*	*2.82E-03*	*4.00E-04*	*3.22E-03*	6.57E-07	8.20E-08	7.39E-07

Table 9.10: Hazard Quotients and cancer risks resulting from exposure to water used for swimming

Lomati Recreator RISK for Water Max value													
Chemical	**Concentration (µg/L)**	**Child Ingestion HQ**	**Child Dermal HQ**	**Child Total HI**	**Adult Ingestion HQ**	**Adult Dermal HQ**	**Adult Total HI**	**Adjusted Ingestion HQ**	**Adjusted Dermal HQ**	**Adjusted Total HI**	**Ingestion Risk**	**Dermal Risk**	**Total Risk**
Atrazine	0.192	2.63E-05	6.55E-05	9.18E-05	5.64E-06	3.83E-05	4.39E-05	9.76E-06	4.37E-05	5.35E-05	2.89E-09	1.29E-08	1.58E-08
Carbofuran	0.0561	4.61E-06	7.11E-06	1.17E-05	9.88E-07	4.16E-06	5.14E-06	1.71E-06	4.75E-06	6.46E-06	-	-	-
Imidacloprid	0.0647	4.43E-07	2.78E-08	4.71E-07	9.50E-08	1.63E-08	1.11E-07	1.64E-07	1.86E-08	1.83E-07	-	-	-
Terbuthylazine	0.02	8.22E-06	8.80E-05	9.62E-05	1.76E-06	5.14E-05	5.32E-05	3.05E-06	5.87E-05	6.18E-05	-	-	-
Thiabendazole	0.0069	2.84E-08	-	2.84E-08	6.08E-09	-	6.08E-09	1.05E-08	-	1.05E-08	-	-	-
*Total Risk/HI	-	*3.96E-05*	*1.61E-04*	*2.00E-04*	*8.49E-06*	*9.39E-05*	*1.02E-04*	*1.47E-05*	*1.07E-04*	*1.22E-04*	2.89E-09	1.29E-08	1.58E-08

Table 9.11: Hazard quotients and cancer risks resulting from ingestion of fish caught in the rivers.

Lomati Resident RISK for Fish Max value			
Chemical	**Surface Water Concentration (mg/L)**	**Ingestion HI**	**Ingestion Risk**
Atrazine	0.000192	3.22E-05	1.11E-07
Carbofuran	0.0000561	6.56E-04	-
Imidacloprid	0.0000647	-	-
Terbutylazine	0.00002	1.53E-04	-
Thiabendazole	6.9E-6	-	-
*Total Risk/HI	-	*8.41E-04*	*1.11E-07*

Table 9.12: Hazard Quotients and cancer risks resulting from exposure to water used for the production of livestock and produce.

Lomati Farmer RISK for Contaminated Water max value							
Chemical	**Soil Concentration (mg/kg)**	**Produce Ingestion HQ**	**Beef Ingestion HQ**	**Milk Ingestion HQ**	**Produce Ingestion Risk**	**Beef Ingestion Risk**	**Milk Ingestion Risk**
Atrazine	0.000192	0.000189	2.45E-09	2.75E-08	5.06E-07	1.72E-11	5.67E-11
Carbofuran	0.0000561	0.00229	1.28E-08	1.44E-07	-	-	-
Imidacloprid	0.0000647	-	-	-	-	-	-
Terbuthylazine	0.00002	0.000119	2.99E-08	3.38E-07	-	-	-
Thiabendazole	0.0000069	-	-	-	-	-	-
*Total Risk/HI	-	*0.0026*	*4.52E-08*	*5.1E-07*	*5.06E-07*	*1.72E-11*	*5.67E-11*

9.3.6 Vals and Renoster Risk Assessment

Table 9.13: Hazard Quotients and cancer risks resulting from exposure to water used for domestic purposes

Vals Renoster Resident RISK for water max value													
Chemical	**Concentration (µg/L)**	**Child Ingestion HQ**	**Child Dermal HQ**	**Child Total HI**	**Adult Ingestion HQ**	**Adult Dermal HQ**	**Adult Total HI**	**Adjusted Ingestion HQ**	**Adjusted Dermal HQ**	**Adjusted Total HI**	**Ingestion Risk**	**Dermal Risk**	**Total Risk**
Alachlor	0.0052	3.32E-05	1.06E-05	4.38E-05	1.42E-05	4.71E-06	1.90E-05	1.80E-05	5.75E-06	2.38E-05	4.33E-09	1.38E-09	5.71E-09
Atrazine	0.35	7.46E-03	9.29E-04	8.39E-03	3.20E-03	4.13E-04	3.61E-03	4.05E-03	5.05E-04	4.55E-03	**1.20E-06**	1.49E-07	**1.35E-06**
Imidacloprid	0.0196	2.09E-05	6.55E-08	2.09E-05	8.95E-06	2.92E-08	8.98E-06	1.13E-05	3.56E-08	1.14E-05	-	-	-
Simazine	0.9	1.15E-02	8.12E-04	1.23E-02	4.93E-03	3.61E-04	5.29E-03	6.25E-03	4.42E-04	6.69E-03	**1.61E-06**	1.14E-07	**1.72E-06**
Terbuthylazine	0.0792	1.27E-03	6.77E-04	1.94E-03	5.42E-04	3.01E-04	8.44E-04	6.87E-04	3.68E-04	1.06E-03	-	-	-
*Total Risk/HI	-	*2.03E-02*	*2.43E-03*	*2.27E-02*	*8.69E-03*	*1.08E-03*	*9.77E-03*	*1.10E-02*	*1.32E-03*	*1.23E-02*	2.81E-06	2.64E-07	3.08E-06

Table 9.14: Hazard Quotients and cancer risks resulting from exposure to water used for swimming

Vals & Renoster Recreational swimming RISK for Surface Water Max value													
Chemical	**Concentration (µg/L)**	**Child Ingestion HQ**	**Child Dermal HQ**	**Child Total HI**	**Adult Ingestion HQ**	**Adult Dermal HQ**	**Adult Total HI**	**Adjusted Ingestion HQ**	**Adjusted Dermal HQ**	**Adjusted Total HI**	**Ingestion Risk**	**Dermal Risk**	**Total Risk**
Alachlor	0.0052	2.14E-07	1.36E-06	1.57E-06	4.58E-08	7.95E-07	8.41E-07	7.93E-08	9.08E-07	9.87E-07	1.90E-11	2.18E-10	2.37E-10
Atrazine	0.35	4.79E-05	1.19E-04	1.67E-04	1.03E-05	6.98E-05	8.01E-05	1.78E-05	7.97E-05	9.75E-05	5.26E-09	2.36E-08	2.88E-08
Imidacloprid	0.0196	1.34E-07	8.42E-09	1.43E-07	2.88E-08	4.92E-09	3.37E-08	4.98E-08	5.62E-09	5.54E-08	-	-	-
Simazine	0.9	7.40E-05	1.04E-04	1.78E-04	1.59E-05	6.10E-05	7.69E-05	2.75E-05	6.97E-05	9.71E-05	7.06E-09	1.79E-08	2.50E-08
Terbuthylazine	0.207	2.13E-05	2.28E-04	2.49E-04	4.56E-06	1.33E-04	1.38E-04	7.89E-06	1.52E-04	1.60E-04	-	-	-
*Total Risk/HI	-	*1.44E-04*	*4.53E-04*	*5.96E-04*	*3.08E-05*	*2.65E-04*	*2.95E-04*	*5.33E-05*	*3.02E-04*	*3.55E-04*	1.23E-08	4.17E-08	5.40E-08

Table 9.15: Hazard quotients and cancer risks resulting from ingestion of fish caught in the rivers.

Vals Renoster RISK for Fish Max value			
Chemical	**Surface Water Concentration (mg/L)**	**Ingestion of fish HQ**	**Ingestion of fish Risk**
Alachlor	5.20E-06	5.08E-06	1.22E-09
Atrazine	0.00035	5.87E-05	2.03E-07
Imidazcloprid	0.0000196	-	-
Simazine	0.0009	4.89E-04	1.26E-07
Terbuthylazine	0.0000792	6.08E-04	-
*Total Risk/HI	-	1.16E-03	3.30E-07

Table 9.16: Hazard Quotients and cancer risks resulting from exposure to water used for the production of livestock and produce.

Vals Renoster Farmer RISK for Contaminated Water max value							
Chemical	**Concentration (mg/kg)**	**Produce Ingestion HQ**	**Beef Ingestion HQ**	**Milk Ingestion HQ**	**Produce Ingestion Risk**	**Beef Ingestion Risk**	**Milk Ingestion Risk**
Alachlor	0.0000052	0.0000129	1.88E-09	2.12E-08	2.41E-09	9.19E-13	3.04E-12
Atrazine	0.00035	0.000344	4.46E-09	5.02E-08	9.22E-07	3.13E-11	1.03E-10
Imidacloprid	0.0000196	-	-	-	-	-	-
Simazine	0.0009	0.00809	2.97E-08	3.37E-07	**1.62E-06**	1.56E-11	5.17E-11
Terbuthylazine	0.000271	0.00162	4.06E-07	0.00000458	-	-	-
*Total Risk/HI	-	*0.0101*	*4.42E-07*	*4.99E-06*	***2.54E-06***	*4.78E-11*	*1.58E-10*

Table 9.17: Hazard quotients resulting from air as a medium of contamination with pesticide

Alachlor	TLV* = 1 mg/m^3		RfD** = 0.01 mg/kg/d	
Downwind distance (m)	Airborne concentration (ng/L) at 1.0 m height worst case	Hazard quotient	Deposition (µg/cm^2) worst case	Hazard Quotient
0	346.08	0.69	1.33	1.21
25	322.12	0.60	0.81	0.74
400	94.02	0.19	0.02	0.02
Atrazine	TLV* = 5 mg/m^3		RfD** = 0.01 mg/kg/d	
Downwind distance (m)	Airborne concentration (ng/L) at 1.0 m height worst case	Hazard quotient	Deposition (µg/cm^2) worst case	Hazard quotient
0	95.06	0.04	0.31	0.28
25	83.27	0.02	0.20	0.18
400	28.24	0.01	0.05	0.05
Simazine	TLV* = 1.65 mg/m^3		RfD** = 0.005 mg/kg/d	
Downwind distance (m)	Airborne concentration (ng/L) at 1.0 m height worst case	Hazard quotient	Deposition (µg/cm^2) worst case	Hazard quotient
0	229.71	0.28	0.99	1.80
25	203.49	0.24	0.62	1.13
400	117.78	0.16	0.099	0.18

**TLV = Threshold Limit Value; ** RfD = Reference dose*

9.3.7 Endocrine disruption

None of the water and sediment samples tested positive for oestrogenic activity making use of the YES assay. The risk assessment therefore focusses on the T47D-KBluc assay. No anti-oestrogenic activity was measured in Letsitele and Lomati, but some activity was determined for water samples collected from Vals River. Cytotoxic activity was measured in four samples from Lomati and seven samples from Vals and Renoster River, but none was found in Letsitele water and sediment samples. The average and maximum oestrogen activity were considered for the current health risk assessment and are summarised in Table 9.18.

Table 9.18: Average and maximum oestrogenic activity of water samples collected from the sampling sites.

Site	**Water EEq (ng/L)**			**Sediment EEq (ng/L)**		
	n	**Average**	**Max**	***n***	**Average**	**Max**
Letsitele	27	0.132	0.580	14	2.705	14.280
Lomati	14	0.073	0.210	5	0.8075	1.91
Vals & Renoster	12	0.395	1.990	6	1.9066	6.65

The average EEq for water samples were below the trigger value of 0.7 ng/L, and only Vals and Renoster study site measured EEqs which suggest further investigation of these sites.

Both cytotoxic and anti-oestrogenic activity was also determined for these sites, it is therefore recommended that the area around the sites be studied and the chemical analysis be examined to possibly identify the sources of oestrogenic contamination. It should always be considered that the trigger value is not a guideline value. Guideline values will need to be set by water administrators after sufficient scientific evidence is presented. Overall, sediment samples measured higher EEqs than water samples which may indicate bioaccumulation of oestrogenic compounds in the sediment. The trigger value is based on the consumption of 2 L of water, assuming an average body weight of 65 kg and an exposure portion of 0.1% and is not a measure of risk for exposure to sediment.

Natural samples consist of a mixture of chemicals which may have (anti)- androgenic and (anti) oestrogenic activity, which could affect the outcome of the assay. Given the complexity of environmental samples (chemical composition, pH etc.), the biological nature of assays and the limitations of sample extraction procedures, results may be underestimated or may even give false negatives.

The androgen activity measured in the study could not be used in a quantitative health risk assessment, as a safe environmental level, or level where no androgenic effects are anticipated in either humans or animals is not available at this stage. However, comparisons can be made between the sites where more (anti-)androgenic activity was measured and water quality at those sites.

As mentioned, there is no standardised method or guideline to assess human health risks associated with endocrine disrupting chemicals (WHO, 2004). Although endocrine disruptors may impact on any one of the hormone driven systems in our body, the bioassays used in the current study, bind to oestrogen or androgen receptors, and can primarily give an indication of the potential disruption chemicals are having on the reproductive system.

9.4 CONCLUSIONS

The predicted health risks based on exposure to pesticides in water is low in each of the case study sites. There are possible health risks associated via the air pathway with inhalation of pesticides close to the point of application as well as via the deposition of pesticides onto soil, and an accidental (or incidental) ingestion of soil.

Every health risk assessment has associated uncertainties. The concentrations of pesticides measured and therefore used in the health risk assessment may not be accurate, in particular if no pesticide was detected (if it was below the detection limit). The health effects that could be considered in the quantitative health risk assessment were limited to excess cancer risks and toxic effects. No dose-response data is available for endocrine effects, but was dealt with through the measurement of both androgenic and oestrogen mimicking and inhibiting effects. On occasion the values measured suggest that possible endocrine disrupting effects were experienced in the Vals- Renoster study site. This is supported by the quantitative study showing higher toxic effects and excess cancer risks based on levels detected in water (whether through direct ingestion of water or through the irrigation of fresh produce). Higher risks were also predicted and calculated via the air pathway. This has helped identify the predominant pathway through which people are exposed to pesticides.

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CHAPTER 10: GENERAL CONCLUSIONS & RECOMMENDATIONS

by

J.M. Dabrowski

CSIR, Natural Resources and Environment, PO Box 395, Pretoria, 0001

The general aim of this project as stipulated in the Terms of Reference in the call for proposals was *"to determine the extent and level of contamination by agricultural chemicals (pesticides, herbicides and plant growth regulants), including Endocrine Disruptive (ED) properties and selected risk assessments for the environment (animal and human health)".*

The project selected three agriculturally intensive catchments (**Chapter 2**) representative of important commercial crops produced in South Africa (i.e. maize, sugar cane, citrus and sub-tropical fruit), where seasonal sampling for pesticides, inorganic chemistry and ED bioassays was conducted in water and sediment. These results were interpreted against guideline values (in the case of inorganic constituents) or subjected to detailed risk assessment methodologies (in the case of pesticides) so as to assess the potential risk of agricultural chemicals to human and animal health. In addition, pesticides were prioritised based on quantity of use, toxicity (to human health) and environmental mobility and their spatial distribution mapped to highlight important source areas (see Volume 2 of this report). These resources were used to prioritise pesticides for monitoring in each of the selected study areas.

This study has provided further evidence of the widespread occurrence of agricultural chemicals in water resources in South Africa (**Chapter 3**). A major limitation associated with the sampling approach adopted in this study is that pesticide contamination in water resources is typically transient, with peak (high) concentrations most often being associated with specific events (i.e. during actual spraying of pesticides or during heavy rainfall events when runoff becomes a major contributing factor). The sampling frequency adopted in all catchments in this study is therefore unlikely to represent peak concentrations. It is however important to note that peak concentrations are likely to be present for relatively short periods of time (a few hours) and exposure of humans and livestock to these peak concentrations through the water pathway is likely to be low. The concentrations detected in these study areas are therefore likely to be representative of a typical exposure scenario.

Despite the monitoring limitations mentioned above this study did however reveal relatively high concentrations of particularly atrazine, terbuthylazine and simazine in maize and sugar cane areas. More importantly these pesticides were detected at similar concentrations over different seasons (wet and dry) indicating that these pesticides have essentially saturated water resources in these catchments. Their ubiquitous presence in water resources warrants further investigation in areas where use is high. In particular, more detailed surveys of groundwater resources and boreholes that deliver drinking water and for human and animal consumption should be surveyed in more detail.

In all study areas, the detection of pesticides was well predicted by indices used in the prioritisation procedure (Volume 2), particularly quantity of use and mobility (as indicated by the GUS index). The frequent detection of atrazine, terbuthylazine and simazine in maize and sugar cane areas is undoubtedly a reflection of their high quantity of use as well as their

high mobility in the environment. Similarly, imidacloprid, which was also highlighted as being highly mobile in the environment, although not detected as frequently, was also found in comparatively high concentrations when detected. Other frequently detected pesticides (e.g. carbofuran, diuron and hexazinone) were also well predicted by outputs from the prioritization procedure (i.e. crop specific use and mobility). These results indicate that indices of use and mobility are very useful in terms of prioritising specific pesticides for detailed monitoring in study areas of interest. In addition, the qualitative screening analysis was also instructive in helping to identify specific pesticides the selected catchment study areas for further quantitative analysis. It is therefore recommended that the combination of these predictive (see Volume 2 of this report) and analytical tools be consulted when planning future pesticide monitoring and risk assessment studies of this nature. Furthermore the pesticide maps developed in Volume 2 of this study provide an additional resource with respect to identifying where in the country contamination of water resources by specific pesticides is likely to occur. Additional useful resources with regards to characterising pesticide use include databases produced by CropLife South Africa (CropLife, 2015) and AgriIntel (AgriIntel, 2015), which provide information on active ingredients and recommended rates of application of registered pesticides for different crops in the country.

The mobility index (GUS index) used in the prioritisation procedure was derived using physicochemical data from international databases. As this data is derived overseas under different environmental conditions, there is often a reluctance to rely on these parameters for modelling pesticide fate and transport in South Africa. This study, together with a number of other studies that have focussed on the fate of pesticides in water resources have however shown that in general, these values provide reasonably accurate indications of pesticide mobility under South African conditions (Dabrowski *et al.*, 2002, Sereda and Meinhardt, 2003; Dabrowski and Balderacchi, 2013). This is supported by research by Meinhardt (2009), which showed that K_{OC} values for pesticides in South African soils were in line with internationally published values and that pesticide adsorption to South African soils may thus be similar. This is an important conclusion given that physicochemical properties of pesticides are an important input parameter with respect to modelling their fate and transport in the environment. The fact that these parameters are often derived in international studies should not therefore be seen as a limitation to their use in modelling studies conducted in South Africa.

Pesticide contamination originates from nonpoint source processes (e.g. leaching, runoff and spray drift) which are which are influenced by a number of environmental and geographical factors and are therefore typically very difficult to identify, quantify and manage. This, together with limitations associated with monitoring of pesticides in the environment (as highlighted above), implies that modelling approaches in exposure assessments are critical with respect to improving our ability to predict the fate, transport and environmental concentrations of numerous pesticides (all with high variation in physicochemical parameters) in the environment. In this respect the good correlation between measured and predicted aerial pesticide concentrations achieved with the AGDISP model (**Chapter 4**) were promising and highlights the applicability and usefulness of this model in understanding the transport of pesticides via spray drift in the environment. While the field monitoring component of the study was a costly and logistically challenging exercise, these studies are important for increasing our confidence in the use of these models for application in risk assessment studies. The application of the AGDISP/AgDrift model is not only limited to

predicting pesticide concentrations in air, but also extends to predicting deposition and resulting concentrations of pesticides in adjacent water bodies. Other studies using simpler empirical spray drift formulae have also shown good correlations between measured and predicted deposition of pesticides in agricultural streams (Schulz *et al*., 2001).

In general, the application of models in risk assessment of pesticides in South Africa is under-utilised. In contrast, countries in Europe and North America rely heavily on runoff, leaching and spray drift models for use in predicting environmental exposure due to pesticides, to the extent that they now form an integral component of the pesticide registration process in these countries. Exposure assessments attempt to quantify the predicted environmental concentration (PEC) of a chemical in the receiving environment under certain environmental conditions and conditions of use (e.g. recommended application rates). The exposure assessment typically relies on fate and transport models, which integrate usage characteristics (e.g. the quantity or rate at which a pesticide is applied) with environmental fate properties of the pesticide (e.g. half-life, solubility etc.) to provide an estimate of the PEC. An exposure assessment can be performed at varying levels of complexity ranging from simple worst-case predictions to more complex predictions that take the environmental fate of pesticides into account as well as environmental conditions (including soil type, climate slope etc.) that influence the movement of pesticides into water resources via runoff, leaching and spray drift.

Results from the exposure assessment are compared to toxicological endpoints derived from hazard assessments, whereby if the PEC exceeds the concentration deemed as hazardous, then an unacceptable risk is likely to exist. For the exposure assessment a tiered system is often employed, whereby a simple worst-case scenario is first adopted (e.g. assuming 10% of the applied quantity of a pesticide moves into and adjacent water resource). If, under these conditions, no risk is expected, then there is no need to use more data intensive procedures. However, if, at this first step a risk cannot be ruled out, then a more detailed assessment takes place, using more realistic data inputs and environmental conditions to perform a more realistic assessment of exposure. A critical component of any modelling procedure is the identification of relevant exposure assessment scenarios that characterise environmental conditions that are used for model input parameters. These conditions vary widely across the country and thus, for the purposes of modelling, exposure assessment scenarios can be developed which are broadly representative of agriculture practiced in major production areas of the country. For example in the EU, 10 different scenarios have been developed for use as data input into modelling, which are representative of typical environmental conditions in major agricultural production areas.

Part of our uncertainty related to the effects of pesticides in the environment relates to the fact that the predicted fate and transport of pesticides in the environment are not considered in the South African pesticide registration process. Currently the DAFF does not possess a mechanism or tools to adequately assess the environmental fate of pesticides under South African conditions. As such the DAFF is unable to estimate or predict the likelihood and quantity of a pesticide that can move into non-target environments (e.g. ground and surface water). Without adequately defining exposure, it is not possible to reliably assess the risk a pesticide poses to the environment. For adoption in South Africa, it is essential that these models are interrogated so as to clearly identify their data requirements and their suitability for performing exposure assessments in South Africa. These requirements need to be assessed against data that is currently available in South Africa to determine the viability of

using these models for risk assessment of pesticides in South Africa. Recent research published by the WRC has investigated potential models for use in South Africa (Jovanovic *et al*., 2012) and further research is required to incorporate the use of these models into routine risk assessment.

Despite this technical knowledge gap, the need for improved management of pesticides in the environment has been echoed by the DAFF (formerly DoA), which in a Pesticide Management Policy for South Africa (RSA, 2006), stated that:

"human health, environmental quality and economic development depend on effective systems that enable South Africans to use pesticides safely and sustainably. Effective systems are those that identify the potential risks of pesticide exposure to the environment and human health and provide government, industry and the community with the right tools to reduce and manage those risks".

The policy also specifically mentions the need to protect water quality through releasing fewer pesticides and/or less toxic pesticides into the environment and, second, to use practices that minimize the movement of pesticides to surface water and groundwater. Improved prioritisation of environmental risk (to inform environmentally friendly use of pesticides), monitoring and modelling approaches are therefore essential to close the gap on assessing the risks of pesticides in the environment. Clearly improved risk assessment at the time of registration would provide a pro-active understanding of risks of a chemical prior to approving its use. At the very least a screening approach identifying highly mobile pesticides and their associated risks should be adopted.

As inorganic chemicals have also been implicated in causing ED effects it is important to include their analysis to establish a baseline against which to interpret the hazards and risks posed by agricultural chemicals (**Chapter 8**). Failure to include these, results in less confidence in interpreting both the bioassay results that may be obtained and exposure assessments. Use of this water quality data is required in order to meaningfully interpret the context of hazards posed by organics and inorganics, without which a differential diagnosis may be difficult to reach. This is presented in greater detail in the Monitoring and Assessment Volume of the WRC Manual on EDCs. While bromide in drinking water was identified as a potential chemical of concern at the farm in Komatipoort where poultry samples were collected, no inorganic chemical was highlighted as being of concern with respect to ED effects in any water samples collected from the three study area.

The ED bioassays used in this study detect chemicals based on their biological activity and determine the total androgenic (**Chapter 5**) and oestrogenic (**Chapter 6**) content of a given sample (Leusch *et al*., 2010). Significant responses in an *in vitro* bioassay should be used as an indicator for further investigation using *in vivo* test models, and/or identification of the active chemical. A negative response would suggest that further investigations might not be warranted. Bioassays therefore play a major role as initial screening tools for environmental samples. ED bioassays conducted on samples collected from many of the study sites showed positive. For many of these samples, pesticides with known EDC effects were detected, although no patterns between ED responses and specific pesticides could be discerned (**Chapter 7**). As ED bioassays are intended for screening purposes it follows that that the detected pesticides may contribute to the observed ED responses. Maximum EEq values were generally well below the trigger value (0.7 ng/L) in the Letsitele and Lomati

catchments. Samples collected in the Vals and Renoster rivers however showed comparatively higher values, with some samples exceeding the 0.7 ng/L trigger value. The frequent detection of atrazine, simazine and terbuthylazine (all known EDCs) in combination with the observed ED bioassay responses highlights this geographical area as a priority for further research, where a more detailed survey of the contamination of human and livestock drinking water resources (surface and groundwater) and associated health risks is recommended. While a trigger value has been recommended there is no standardised method or guideline to assess human health risks associated with endocrine disrupting chemicals, an area which requires further research.

It is important to note that mixtures of agricultural chemicals (as observed in most samples collected in this study) may result in additive, synergistic or antagonistic responses , implying that the expected ED bioassay response may be higher or lower than anticipated from known responses of individual chemicals (Chen *et al*., 2007; Haas *et al*., 2008). Furthermore, it is also possible that other contaminants with ED properties (e.g. detergents, pharmaceuticals and natural and synthetic oestrogens in sewage effluent) were also present in the samples which caused the observed ED bioassay responses. In particular, the relatively high ED results observed at VL2 may be indicative of other sources of contamination originating from the town of Kroonstad, including industrial and sewage effluent. Furthermore, results from the spray drift study indicate that inhalation of airborne pesticide levels potentially poses a greater risk to human health than those derived from use of water resources. In this respect a comparative study of the relative importance of different sources of EDCs in the environment is recommended to prioritise and focus future research initiatives in this field. The widely acknowledged poor state of many of the country's sewage treatment works may for example highlight this source as a far more important contributor to ED effects in water resources than agricultural chemicals (especially considering the continuous versus transient nature of contamination originating from sewage treatment works and agriculture, respectively).

The predicted cancer and toxicity risks based on exposure to pesticides in water in each of the case study sites is low (**Chapter 9**). This finding should however be interpreted based on the limitations of the sampling design discussed above. Given the inherent risks associated with pesticide exposure, in combination with the fact that this and many other studies have shown that pesticides regularly occur in surface and ground water that is used for human consumption and livestock watering, provides strong justification for the development of risk-based domestic and livestock use water quality guidelines that include priority pesticides used in the country. Indices of use, toxicity and mobility (see Volume 2 of this report) could be used to prioritise pesticides for which water quality guidelines should be developed. Despite the low hazards posed by pesticides detected in this study, the increased recognition of ED effects from exposure to low concentrations and complexities around concurrent exposure to multiple potential hazards via the water pathway, a precautionary approach is still justified.

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APPENDICES

APPENDIX 1: PESTICIDES INCLUDED IN QUALITATIVE SCREENING

Table A1: List of pesticide compounds included in the qualitative screening analysis

Abamectin	Endosulfan II (Beta)	Naled
Ac DON 3	Endosulfan Sulfate	Nicosulfuron
Acephate	Endrin	Nitenpyram
Acetamiprid	Endrin Aldehyde	NIV
Afla B1	Endrin Ketone	Omethoate
Afla B2	Epoxiconazole	OTA
Afla G1	EPTC	Oxadixyl
Afla G2	Esprocarb	Oxamyl
Alachlor	Ethiofencarb	Oxydemeton methyl
Alanycarb	Ethiofencarb-sulfone	Paclobutrazol
Aldicarb	Ethiofencarb-sulfoxide	Parathion
Aldicarb Sulfoxide	Etofenprox	Parathion-methyl
Aldicarb Sulphone	Etoxazole	PCP
Aldicarb-sulfoxide	Famoxadone	Penconazole
Aldrin	Famphur	Pencycuron
Alpha-BHC	Fenazaquin	Pendimethalin
Alpha-Chlordane	Fenbuconazole	Phenmedipham
Ametryn	Fenhexamid	Phenthoate
Aminocarb	Fenobucarb	Phorate
Atrazine	Fenoprop (2,4,5-T)	Phorate sulfoxide
Azinphos methyl	Fenothiocarb	Phosalone
Azinphos-ethyl	Fenoxap-p-ethyl	Phoxim
Azoxystrobin	Fenoxycarb	Picoxystrobin
Benalaxyl	Fenpropimorph	Pirimicarb
Bendiacarb	Fenpyroximate	Pirimiphos-ethyl
Benfuracarb	Fenthion	Pirimiphos-methyl
Benomyl	Fenuron	Prochloraz
Bentazon	Flazasulfuron	Promecarb
Beta-BHC	Florosulam	Prometon
Bitertanol	Fluazifop	Prometryn
Boscalid	Fluazifop-p-butyl	Propachlor
Bromacil	Fluazinam	Propamocarb
Bromoxynil	Fludioxonil	Propamocarb HCl
Bromuconazole	Flufenacet	Propanil
Bupirimate	Flufenoxuron	Propaquizafop
Buprofezine	Flumeturon	Propargite
Butocarboxim	Fluometuron	Propazine
Butocarboxim-sulfoxide	Fluoxypyr	Propiconazole
Butoxycarboxim	Fluroxypyr	Propoxur
Cadusafos	Flusilazole	Propyzamide

CAP	Fosthiazate	Prosulfuron
Carbaryl	Furathiocarb	Pyraclostrobin
Carbendazim	FUS X	Pyributicarb
Carbetamide	Gamma-BHC	Pyridate
Carbofuran	Gamma-Chlordane	Pyrimethanil
Carbosulfan	Haloxyfop	Pyriproxyfen
Carboxin	Haloxyfop methyl	Quinmerac
Chlorfenvinphos	Heptachlor	Quinoxyfen
Chloridazon	Heptachlor Epoxide Isomer B	Quizalofop-p-ethyl
Chloropropham	Heptenophos	Resmethrine
Chlorotoluron	Hexaconazole	Rimsulfuron
Chlorpyrifos	Hexaflumuron	Sebuthylazine
Chlorsulfuron	Hexazinone	Sethoxydim
Chlortoluron	Hexythiazox	Siduron
Clofentezine	HT 2	Simazine
Clomazone	Hydroxycarbofuran 3-	Simetryn
Clopyralid	Imazalil	Spinosad A
Clothianidin	Imazamox	Spinosad D
Coumaphos	Imazapyr	Spiroxamin
Cyanazine	Imidacloprid	Sulfotep
Cymoxanil	Indoxacarb	Sulprofos
Cyproconazole	Ioxynil	T2
Cyprodinil	Iprodione	Tebuconazole
D 2,4-	Iprovalicarb	Tebufenozide
DAS	Isofenfos	Tebufenpyrad
DB 2,4-	Isoprocarb	Tebuthiuron
DDD 4,4'-	Isoproturon	Teflubenzuron
DDE 4,4'-	Isoxaflutole	Tepraloxydim
DDT 4,4'-	Lenacil	Terbucarb
Delta-BHC	Linuron	Terbuthialzine
Demeton-S-methyl	Lufenuron	Terbuthialzine desethyl
Demeton-S-methyl-sulfone	Malaoxon	Terbuthylazine
desethyl atrazine	Malathion	Terbutryn
Desisopropyl atrazine	MCPA	Tetrachlorvinphos
Desmedipham	MCPB	Tetraconazole
Diazinon	MCPP (Mecoprop)	Thiabendazole
Dicamba	Mecarbam	Thiacloprid
Dichlorvos	Mepanipyrim	Thiamethoxam
Dicrotophos	Metalaxyl	Thidiazuron
Dieldrin	Metamitron	Thiobencarb
Difenoconazole	Metazachlor	Thiodicarb
Diflubenzuron	Methabenzthiazuron	Thiofanox
Diflufenican	Methamidophos	Thiofanox-sulfone
Dimefuron	Methiocarb	Thiophanate methyl

Dimepiperate	Methiocarb-sulfone	Thiuram
Dimethachlor	Methomyl	Tokuthion
Dimethenamide	Methoxychlor	Tolylfluanid
Dimethoate	Methoxyfenozide	TP 2,4,5-
Dimethomorph	Metobromuron	Triadiminol
Dinoterb	Metolachlor	Triallate
Dioxacarb	Metosulam	Triazophos
Diphenylamine	Metoxuron	Trichlorofon
Disulfoton	Metribuzin	Triclopyr
Diuron	Mevinphos	Trifloxystrobin
Dodine	Monocrotophos	Triflumuron
DON	Monolinuron	Triforine
DP 2,4-	Monuron	XMC
Endosulfan I (Alpha)	Myclobutanil	ZON

APPENDIX 2: AGDISP MODEL INPUTS AND EXPERIMENTAL RESULTS

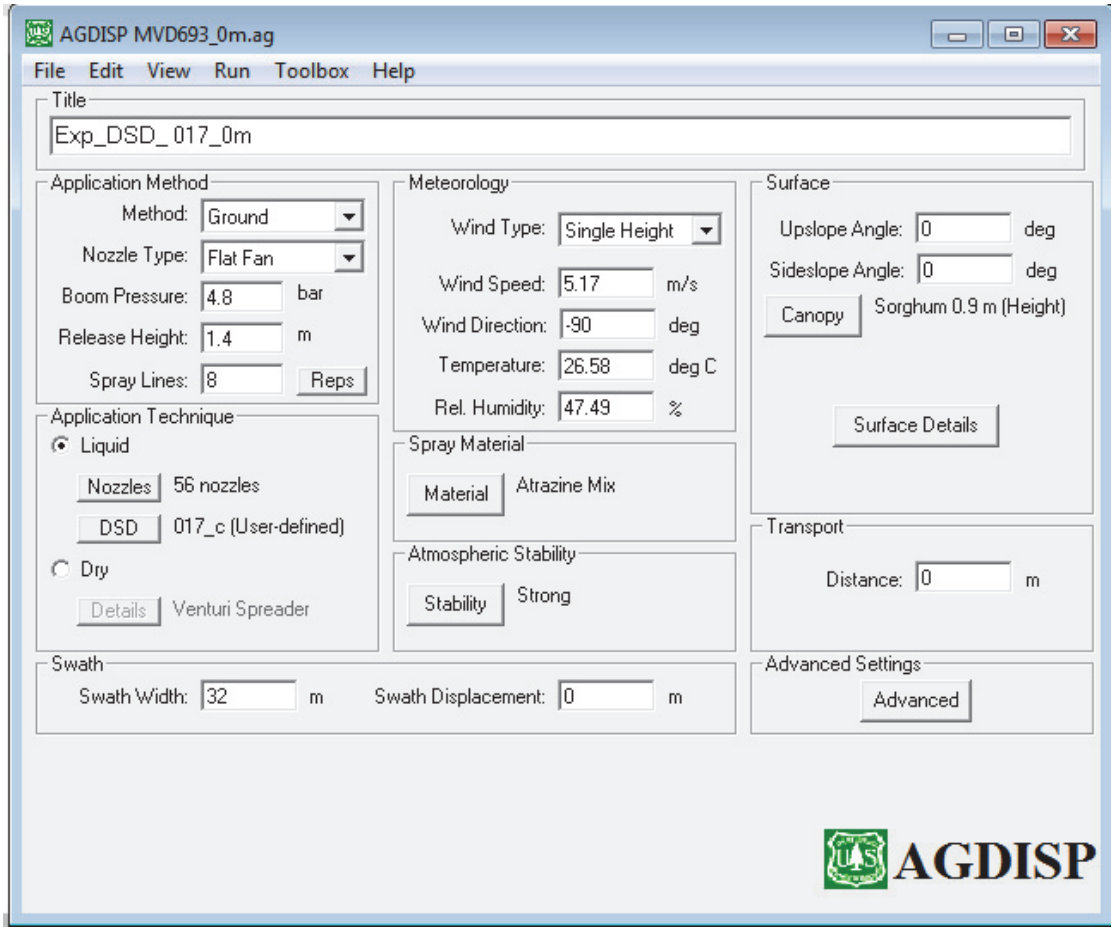

Figure A1: The graphic user interface (GUI) of the AGDISP model software showing the input parameters needed to run the model

Table A2: Summary of the AGDISP input parameters using a droplet size distribution with 166 µm average diameter

AGDISP MVD693_0m.ag 8.27 10-29-2014 12:41:10 Page 1

AGDISP Input Data Summary

Title: Exp_DSD_ 017_0m
Notes:

Calculations Done: Yes
Run ID: AGDISP MVD693_0m.ag 8.27 10-29-2014 12:41:10

--APPLICATION METHOD--	
Method	Ground
--Ground Sprayer--	
Nozzle Type	Flat Fan
Boom Pressure (bar)	4.8
--Spray Lines--	
Release Height (m)	1.4
Spray Lines	8
Optimize Spray Reps	No

Spray Line Reps

#	Reps
1	1
2	1
3	1
4	1
5	1
6	1
7	1
8	1

--APPLICATION TECHNIQUE--	
Application Technique	Liquid
--Nozzles--	
Boom Length (%)	85.94

Nozzle Locations

#	Hor(m)	Ver(m)	Fwd(m)
1	-13.75	0	0
2	-13.25	0	0
3	-12.75	0	0
4	-12.25	0	0
5	-11.75	0	0
6	-11.25	0	0
7	-10.75	0	0
8	-10.25	0	0
9	-9.75	0	0
10	-9.25	0	0
11	-8.75	0	0
12	-8.25	0	0
13	-7.75	0	0
14	-7.25	0	0
15	-6.75	0	0
16	-6.25	0	0
17	-5.75	0	0
18	-5.25	0	0
19	-4.75	0	0
20	-4.25	0	0
21	-3.75	0	0
22	-3.25	0	0
23	-2.75	0	0
24	-2.25	0	0
25	-1.75	0	0
26	-1.25	0	0
27	-0.75	0	0
28	-0.25	0	0
29	0.25	0	0
30	0.75	0	0
31	1.25	0	0
32	1.75	0	0
33	2.25	0	0
34	2.75	0	0

35	3.25	0	0
36	3.75	0	0
37	4.25	0	0
38	4.75	0	0
39	5.25	0	0
40	5.75	0	0
41	6.25	0	0
42	6.75	0	0
43	7.25	0	0
44	7.75	0	0
45	8.25	0	0
46	8.75	0	0
47	9.25	0	0
48	9.75	0	0
49	10.25	0	0
50	10.75	0	0
51	11.25	0	0
52	11.75	0	0
53	12.25	0	0
54	12.75	0	0
55	13.25	0	0
56	13.75	0	0

--Drop Size Distribution--

Name	017 c
Type	User-defined

Drop Categories #	Diam (um)	Frac
1	48.74	0.0010
2	108.59	0.0168
3	168.44	0.0381
4	228.29	0.0195
5	288.14	0.0336
6	348.00	0.0593
7	407.85	0.0477
8	467.70	0.0480
9	527.55	0.0000
10	587.40	0.0475
11	647.25	0.0636
12	707.11	0.1657
13	766.96	0.0000
14	826.81	0.1325
15	886.66	0.3268

--SWATH--

Swath Width	32 m
Swath Displacement	0 m

--METEOROLOGY--

Wind Speed (m/s)	5.17
Wind Direction (deg)	-90
Temperature (deg C)	26.58
Relative Humidity (%)	47.49

--SPRAY MATERIAL--

Name	Atrazine Mix
Spray Material Evaporates	Yes
Spray Volume Rate (L/ha)	150
Active Fraction	0.003
Nonvolatile Fraction	0.003
Active Fraction of Tank Mix	0.003
Fraction of Active Solution that is Nonvolatile	1
Additive Fraction of Tank Mix	0
Fraction of Additive Solution that is Nonvolatile	1

--ATMOSPHERIC STABILITY--

Atmospheric Stability	Strong

--SURFACE--	----------
Upslope Angle (deg)	0
Sideslope Angle (deg)	0
--Canopy--	----------
Type	Height
Name	Sorghum
Height (m)	0.9
--TRANSPORT--	----------
Flux Plane Distance (m)	0
--ADVANCED SETTINGS--	----------
Wind Speed Height (m)	2
Max Compute Time (sec)	600
Max Downwind Dist (m)	400
Vortex Decay Rate (OGE) (m/s)	0.15
Vortex Decay Rate (IGE) (m/s)	0.56
Aircraft Drag Coeff	0.1
Propeller Efficiency	0.8
Ambient Pressure (mb)	1013
Ground Reference (m)	0
Save Trajectory Files	No
Half Boom	No
Default Swath Offset	1/2 Swath
Specific Gravity (Carrier)	1
Specific Gravity (Active/Additive)	1
Evaporation Rate (μm^2/deg C/sec)	84.76

Table A3: PUF atrazine results from GC-NPD analysis

Downwind distance (m)	Vertical height (m)	Ret. Time (Min)	Area (pA*s)	Amount (pg/uL)	Extract (mL)	Amount extract (pg)	Flow Rate (L/min)	Sampled air (L)	Conc. in sampled air (ng/L)
10	0.5	8.262	10.980	378.73	250.00	94683745.00	375	22500	4.21
	1.0	8.261	13.027	445.44	250.00	111360442.50	410	24600	4.53
	1.5	8.262	9.711	337.36	250.00	84339975.00	350	21000	4.02
	2.0	8.262	9.609	334.03	250.00	83507697.50	440	26400	3.16
25	0.5	8.263	8.364	293.47	200.00	58693410.00	350	21000	2.80
	1.0	8.261	16.982	574.31	200.00	114861780.00	425	25500	4.50
	1.5	8.263	10.777	372.11	200.00	74422672.00	330	19800	3.76
	2.0	8.264	8.533	298.99	200.00	59797044.00	350	21000	2.85
50	0.5	8.262	13.541	462.17	100.00	46217260.00	350	21000	2.20
	1.0	8.261	12.449	426.60	100.00	42660397.00	320	19200	2.22
	1.5	8.261	12.461	426.99	100.00	42699075.00	330	19800	2.16
	2.0	8.261	13.736	468.54	100.00	46853611.00	350	21000	2.23
100	0.5	8.263	4.802	177.38	100.00	17738150.00	250	15000	1.18
	1.0	8.262	5.867	212.11	100.00	21210590.00	260	15600	1.36
	1.5	8.262	5.477	199.37	100.00	19937050.00	220	13200	1.51
	2.0	8.262	3.161	123.90	100.00	12390007.00	250	15000	0.826
200	0.5	8.261	2.797	112.06	100.00	11206177.00	350	21000	0.534
	1.0	8.263	4.044	152.70	100.00	15269627.00	350	21000	0.727
	1.5	8.262	3.115	122.41	100.00	12240566.00	300	18000	0.680
	2.0	8.263	3.406	131.89	100.00	13188514.00	315	18900	0.698
400	0.5	8.260	19.541	657.69	5.00	3288468.10	350	21000	0.157
	1.0	8.266	22.965	769.29	5.00	3846469.55	350	21000	0.183
	1.5	8.268	23.536	787.89	5.00	3939456.50	350	21000	0.188
	2.0	8.260	14.697	499.85	5.00	2499269.55	300	18000	0.139

APPENDIX 3: EXPOSURE PARAMTERS USED IN HUMAN HEALTH RISK ASSESSMENT

Table A4: Exposure parameters for exposure to contaminants in water and soil from RAIS (2013)

Abbreviation	Variable	Unit	Value
EF	Exposure frequency	day/year	350
ED	Exposure duration	years	30
Cw	Concentration of contaminant in water	mg/L	
Lt	Lifetime	years	70
CF_p	contaminated intake fraction		1
BW_a	Body weight adult	kg	70
BW_c	Body weight child	kg	15
ED_c	exposure duration child	years	6
IRW_a	water intake rate - adult	L/day	2
IRW_c	Water intake rate - child	L/day	1
IRW_{rec}	Water intake from recreational use	L/day	0.05
IRF	Fish intake rate	g/day	25
K	Volatilization factor of Andelman	L/m^3	0.5
BCF	Bioconcentration factor		
IRP_{Vg-a}	vegetable intake rate - adult	mg/day	28500
IRP_{vg-c}	vegetable intake rate - child	mg/day	10400
IRP_{fr-a}	fruit intake rate - adult	mg/day	56200
IRP_{fr-c}	fruit intake rate - child	mg/day	14800
IRM_a	Milk ingestion rate - adult	mg/day	614000
IRM_c	Milk ingestion rate - child	mg/day	265000
IRB_a	Beef ingestion rate - adult	mg/day	138000
IRB_c	Beef ingestion rate - child	mg/day	12900
IRP_{fr-adj}	age adjusted fruit intake rate	mg^{-year}/kg-day	25188.57
IRP_{vg-adj}	age adjusted vegetable intake rate	mg-year/kg-day	13931.43
IRM_{adj}	age adjusted milk intake rate	mg-year/kg-day	316514.3
IRB_{adj}	age adjusted beef intake rate	mg-year/kg-day	52474.29
I_r	Irrigation rate	L/m^2 - day	3.62
F	Irrigation period		0.25
T_b	long term deposition and buildup)	day	10950
$Lamda_{HL}$	soil leaching rate	1/day	0.000027
P	area density for root zone	kg/m^2	240
MLF_p	produce plant mass loading factor		0.26
I_f	Interception fraction		0.42
T	Translocation factor		1
t_w	weathering half-lie	day	14
t_y	above ground exposure time	day	60
Y_y	Plant yield - wet	kg/m^2	2
Q_w -dairy	dairy water intake rate	L/day	92

Abbreviation	Variable	Unit	Value
Q_w - beef	beef water intake rate	L/day	53

Table A5: Reference dose and carcinogenicity parameters used in risk calculations

Chemical	RfD (mg/kg/d)	Oral slope factor $(mg/kg/d)^{-1}$	Inhalation unit risk per mg/m^3
Alachlor	0.01	0.056	
Atrazine	0.035	0.23	
Carbofuran	0.005		
Diphenylamine	0.025		
Imazalil	0.013		
Propiconazole	0.013		
Simazine	0.005	0.12	
Terbutryn	0.001		
Thiazole			0.00066

APPENDIX 4: CAPACITY BUILDING & KNOWLEDGE DISSEMINATION

CAPACITY BUILDING

M.Sc Dissertations

All M.Sc students and their dissertation listed below have contributed directly to the objectives of the project.

Castleman BA (Graduated 2013):

Investigation of the contamination of water resources by agricultural chemicals and their impact on environmental health in the Letsitele Valley.

Centre for Environmental Studies, University of Pretoria

Nsibande SA (Submitted 2015):

Pesticide spray drift monitoring in the evaluation of air dispersion models: A South African atrazine case study.

Department of Chemistry, University of Pretoria.

Powrie L (In progress):

Determining the (anti)androgenic activity of agricultural pesticides in water systems with a luminescence bioassay.

Unit of Environmental Sciences and Management, North West University.

Staff and Student Development

Environmental Chemical Pollution and Health Research Unit, University of Pretoria

Staff and research assistants were involved in the project, where they were taught specific laboratory techniques.

2011:

Ms S Van Wyk (staff) -	Solid phase extraction procedure, yeast oestrogen screen (YES) and T47D-KBluc bioassay methods
Ms A Swanepoel (research assistant) -	Sample preparation methodology, cell culture principles and media preparation

2012:

Ms Al Swanepoel (research assistant) -	Solid phase extraction procedures, YES and T47D-KBluc bioassay methods

2013:

Ms N Vermeulen (research assistant) -	Sample preparation methodology, solid phase extraction procedures, cell culture principles, media preparation and YES bioassay method
Mr SM Patrick (research assistant) -	Sample preparation methodology, solid phase extraction procedures, cell culture principles, media preparation and YES bioassay method

2014:

Ms J du Plooy (research assistant) -	Sample preparation methodology, solid phase extraction procedures, cell culture principles, media preparation and YES and T47D-KBluc bioassay methods

KNOWLEDGE DISSEMINATION

Paper accepted for publication:

Nsibande S, Dabrowski JM, Van Der Walt E and Forbes P (2015). Validation of the AGDISP model for predicting airborne spray drift of atrazine in South Africa. *Chemosphere* **138*:*** 454-461.

Papers in preparation:

Nsibande SA, van der Walt E, Swanepoel L, Forbes PBC (2015). Direct sample analysis-time of flight mass spectrometry for rapid pesticide deposition determinations.

Oral Conference Presentations:

Meyer JA* (2014). Water Committee Position Statement 2014. Premier Pork Producers AGM. Emperors Palace Conference Centre, Kempton Park, 3 September 2014 *(*Invited Speaker)*.

Meyer JA* (2015). The effect of water quality on feedlot performance and compliance monitoring. SA Feedlot Conference 2015, KievitsKroon Conference Venue, Roodeplaat, 12 March 2015 *(*Invited Speaker)*.

Nsibande SA*, Dabrowski JM, van der Walt E and Forbes PBC (2014). Atrazine spray drift monitoring for model evaluation. Analitika Conference, Parys, 7-11 September 2014 *(*SA Nsibande won the ChromSA Best Student Oral presentation prize for this presentation*)

Nsibande SA, Dabrowski JM, van der Walt E, Venter A and Forbes PBC* (2014). Air dispersion modelling: a South African pesticide validation case study. National Association for Clean Air Conference, Umhlanga, Durban, 8-10

October 2014 *(*PBC Forbes won the Best Scientific Paper prize at the conference for this presentation*).

Nsibande SA, Dabrowski JM, van der Walt E and Forbes PBC (2014). Atrazine spray drift monitoring for model validation. SACI Postgraduate Student Symposium, Limpopo Region, 23 October 2014.

Nsibande SA, Dabrowski JM, van der Walt E and Forbes PBC (2014). Atrazine spray drift monitoring for model validation. SACI Postgraduate Student Symposium, University of North-West, Potchefstroom, 11 November 2014.

Powrie L & Pieters R. (2013). Die gebruik van 'n luminisensie bio-analise om die (anti)androgeen aktiwiteit van landouplaagdoders in akwatiese stelsels te bepaal. SA Academy of Science and Art Natural Sciences Conference, University of Pretoria, 7-8 November.

Poster Conference Presentations:

Nsibande SA*, Swanepoel L and Forbes PBC (2014). How fast is fast? Using DSA-TOF MS for pesticide drift sample screening. Analitika Conference, Parys, South Africa, 7-11 September 2014. *(*SA Nsibande won the SAAMS Best Student Poster award)*.

Nsibande SA*, Swanepoel L and Forbes PBC (2014). How fast is fast? Using DSA-TOFMS for pesticide drift sample screening. National Association for Clean Air Conference, Umhlanga, Durban, 8-10 October 2014.

Nsibande SA, Dabrowski JM, van der Walt E, Venter A and Forbes PBC* (2014) Comparative pesticide air monitoring in validation of air dispersion models. 16th IUAPPA World Clean Congress, Cape Town, South Africa, September 2013.

MSc Dissertation Abstracts

INVESTIGATION OF THE CONTAMINATION OF WATER RESOURCES BY AGRICULTURAL CHEMICALS, AND THEIR IMPACT ON ENVIRONMENTAL HEALTH IN THE LETSITELE VALLEY

Barbara A. Castleman

Promoter: Dr Patricia B.C. Forbes
Department: Chemistry
Faculty: Faculty of Natural and Agricultural Sciences
University: University of Pretoria
Degree: Master of Science

The agricultural sector is highly developed in South Africa, with agrichemicals being used extensively to improve yields and ensure that crops are of high quality for market. Inappropriate application of agrichemicals may affect non-target communities and ecosystems, specifically aquatic ecosystems, due to leaching into groundwater or run-off into surface water. In rural areas, many communities do not have access to clean piped water, and thus collect water directly from the resource.

Pesticides residues found in water resources are generally water soluble compounds which are being used extensively in the area, although levels are usually fairly low. However, current opinion on chronic health problems is that it is likely to be as a result of long term exposure to pesticides at low concentrations. Rural communities are more likely to experience sub-acute exposure through environmental routes, making monitoring for pesticides in rural areas as well as the prioritisation of pesticides according to their carcinogenic and endocrine disrupting effects, important.

This study forms part of the Water Research Commission study, Project Number K5/1956, which is focusing on the exposure of humans and animals to pesticides through their interaction with and use of environmental resources on which they depend. This study focuses on only the Letsitele area, rather than a variety of areas, and looks at the chemistry of the priority pesticides detected in this area, in order to determine exposure parameters such as solubility and persistence in the environment. The Letsitele valley in Limpopo Province was selected for this study due to the area being regarded as an important agricultural area in the Lowveld, supporting a prosperous commercial farming community as well as a significant amount of subsistence farming. There is no industry in the area and so chemical pollution is expected to come from agricultural activities through the use of agrochemicals. Ten sampling sites were selected from approximately 15 km of the Letsitele River and its tributaries; between commercial and subsistence farming and residential areas. Three of the sites were drinking water taps in the residential areas supplied by boreholes.

Sampling was conducted during different seasons to compare the different flows and seasonality of pesticide applications. Eleven pesticides in total were detected in both the water and sediment, with the insecticide imidacloprid ($\leq 4.0 \times 10^{-3}$ ppb) and

fungicides such as propiconazole (≤ 3.8 x 10^{-3} ppb) and thiabendazole (≤ 3.6 x 10^{-2} ppb) being the most frequently detected. The majority of the pesticides detected were those that are purchased for use on crops in the vicinity, including imidacloprid and thiabendazole, but there were also those that were not expected, such as flusilazole and diphenylamine (≤ 2.7 x 10^{-2} ppb), as they are not registered for use on the crops grown in the area. In terms of the chemical prioritisation, six of the eleven pesticides detected in the study area do feature in the top fifty priority pesticides. The environmental properties of pesticides most likely to cause pollution of groundwater and surface water are water solubility, mobility and persistence. The majority of the pesticides detected have low aqueous solubility, but soil half-life values above 60 days, with four above 200 days, which means that they can be considered to be highly persistent in the soil and, therefore, a potential hazard to the environment and to the health of the community. Most are also stable to hydrolysis, implying persistence in water unless susceptible to photolysis, and a potential concern should they reach groundwater. There were a number of pesticides detected in water from the borehole taps sampled including diphenylamine (≤ 2.7 x 10^{-2} ppb), imidacloprid (≤ 3.0 x 10^{-4} ppb), thiabendazole (≤ 3.6 x 10^{-2} ppb) and carbendazim, which is concerning, especially the latter as it has a fairly high toxicological score. The majority of concentrations measured were not very high in terms of ppb, with all below the European Union (EU) set maximum of 0.1 µg/L, i.e. 0.1 ppb for individual pesticides, and the total pesticide concentration for each site, below the 0.5 µg/L limit. Site 4, during November 2011, was the worst case scenario with a total of 0.127 µg/L, although this total excludes carbendazim and flusilazole which were not included in the quantitative analyses. The combination of these pesticides is concerning as the synergistic effect of the pesticides is unknown. However, when considering the number of pesticides that are purchased nationally for use on the crops grown in the Letsitele area, 100 in total, detecting only eleven pesticides in both water and sediment is encouraging for the environment and the community.

PESTICIDE SPRAY DRIFT MONITORING IN THE EVALUATION OF AIR DISPERSION MODELS: A SOUTH AFRICAN ATRAZINE CASE STUDY.

Sifiso A. Nsibande

Promoter: Dr Patricia B.C. Forbes
Department: Chemistry
Faculty: Faculty of Natural and Agricultural Sciences
University: University of Pretoria
Degree: Master of Science

Air dispersion software models that evaluate pesticide spray drift during application have been developed. These models can potentially serve as a cheaper and more convenient alternative to field monitoring campaigns. Such models require validation against field monitoring data in order for them to be employed with confidence, especially when they are used to implement regulatory measures or to evaluate potential human exposure levels.

In this project, a pesticide active ingredient, namely atrazine, was used as a tracer to monitor spray drift up to 400 m downwind for comparison and validation of the AGricultural DISPersal (AGDISP) model outputs. Airborne drift samples were collected using high volume air sampling onto polyurethane foam (PUF) at six downwind locations while ground deposition drift was captured with chromatography fallout paper samplers. Additional data, including meteorological information and some application parameters required to simulate spray drift with AGDISP, was collected.

Airborne samples were extracted with a plunger method using a hexane:acetone solvent mixture and analysed by Gas Chromatography coupled to a Nitrogen Phosphorus Detector (GC-NPD) which performed well (94.5% recovery, 3.3% RSD and LOD 8.7 pg). Atrazine airborne concentrations ranged from 4.55 ng L^{-1} adjacent to the field to 186 pg L^{-1} at 400 m downwind. The experimental results correlated favourably with the modelled output, suggesting that the AGDISP model can be used to provide a good estimate for airborne drift in risk assessment studies or for regulatory purposes.

A simple and rapid screening method using a Direct Sample Analyser coupled to a Time-of-Flight Mass Spectrometer (DSA-TOFMS) was employed for semi-quantitation of atrazine deposition. This method was shown to be quick (30 min extraction and 25 s analysis) and useful for the large sample set that was collected. The deposition sample extracts were also analysed by GC-NPD using a method similar to that used for airborne samples. Compared to the AGDISP-simulated deposition output, the model under-predicted the deposition by up to one order of magnitude compared to the GC-NPD results and even more compared to the DSA-TOFMS results. This suggested that the model should be used cautiously for predicting pesticide deposition.

For the first time this project has shown the use of a pesticide active ingredient to validate the AGDISP ground application model under local South African conditions up to 400 m downwind of the application area.