EXECUTIVE SUMMARY

Introduction

A prototype implementation manual for the national microbial monitoring programme (NMMP) for groundwater was completed in 2004 [Murray et al., 2004a]. That mainly desktop study produced a general framework for the design of the monitoring programme. Before the design can be formally adopted by the Department of Water Affairs and Forestry (DWAF), the core design and a number of specific issues contained therein needed to be tested.

This report describes the choice of pilot study sites, the location and drilling of monitoring boreholes, the data collected and the assessment thereof. It also includes an examination and refinement of sampling and analysis protocols for bacteria and viruses. The initial version of this report served as a preliminary "implementation manual" for this pilot study (supplementing the main manual). This final version records the refinements required for the final implementation manual aimed at full-scale implementation.

Project objectives

The project objectives as indicated in the original project proposal are as follows:

- To develop improved guidelines for defining faecal pollution attenuation zones in groundwater down-gradient of significant pollution sources.
- To test the core design of the monitoring programme using *Escherichia coli* (*E. coli*) as the primary monitoring variable.
- To produce a refined implementation manual for the national microbial monitoring programme for groundwater.

In order to achieve the objectives of the NMMP, monitoring viruses will also be important. However, a design framework for viruses was not included in the original design. Accordingly, a separate project was created to run concurrently with the present one to address this. This had the following objective:

- To develop detailed virus monitoring protocols for the national monitoring programme for inclusion in a refined implementation manual.

Monitoring objectives and design philosophy

The objectives of the NMMP for groundwater are as follows [Murray et al., 2004a]:

National Microbial Monitoring Programme for Groundwater
National Objectives

To measure, assess and report on a regular basis
the status and trends
of the microbial water quality
that reflects the degree of faecal pollution
(because of the associated human health risks)
of South African groundwater resources
in a manner that is
soundly scientific and
that will support strategic management decisions
in the context of sustainable fitness for use of those water resources.

In essence, the philosophy of the design of the monitoring programme focuses on monitoring faecal contamination of groundwater in a manner in which local containment of the contamination can be confirmed. If containment is indeed observed, then statements, although cautious, can be made about the aquifer down-gradient of the "containment" borehole perhaps being uncontaminated. In this way it is hoped that general statements can be made about the status of aquifers without having to monitor the aquifer at many points. The intention is to maximise information and minimise costs because the NMMP is a national programme.

Pilot study focal issues

To achieve the above project objectives, the following four specific issues were identified:

1. Suitability of potassium as an indicator of flow path.
2. Distance to containment borehole.
3. Presence of viruses in containment borehole in the absence of *E. coli*.
4. Suitability of *E. coli*.

Issues 1 and 2 relate directly to the first project objective above, namely to develop improved guidelines for defining the faecal pollution attenuation zones. Issue 3 relates directly to a fundamental suspicion that viruses, being smaller than bacteria, may travel further than bacteria in groundwater. This is an important issue for the project objective relating to virus monitoring protocols. Issue 4 was investigated simply by comparing the occurrence of *E. coli* with that of enterococci because the latter is known to live longer in the environment (although the environment itself can be a source of enterococci, besides the faecal contamination in the pollution sources studied).

Monitoring sites

Three sites were chosen:

- Ogies sewage treatment works, 85 km south east of Pretoria on a fractured hard-rock aquifer.
    - A background borehole was constructed up-gradient of the pollution source.
    - Based on the down-gradient topography the groundwater flow direction was estimated and a series of three boreholes (“source”, “middle” and “containment”) were constructed along this direction.
    - After seven months of monitoring it was noted that *E. coli* had only been detected once. This was unexpected since the source borehole is closest to the pollution source. It was assumed that sampling was taking place too deep.
    - Another borehole was therefore constructed and sampling took place at a shallower depth. However, only three months data were obtained before the end of the sampling period.
- Brits water care works, 47 km north west of Pretoria on a fractured hard-rock aquifer.
    - A background borehole was again constructed up-gradient of the pollution source. Faecal contamination of the ground surface in the vicinity of the background borehole was visible.
    - Based on the down-gradient topography the groundwater flow direction was estimated and a series of three boreholes (“source”, “middle” and “containment”) were constructed along this direction. However, the topographical gradient was very low.
    - Problems were experienced with both the middle and containment boreholes. Below about 12 m and 10 m respectively there was evidence of an alluvial aquifer (inevitably associated with the nearby Crocodile river).

- Potsdam sewage treatment works, 13 km north east of Cape Town on a coastal unconsolidated sand aquifer.
  - Two down-gradient wellpoints were constructed using water jetting.
  - The groundwater flow direction was indicated by the on-site manager on the basis of a previous study that had been performed in the area.

Monitoring variables

The bacteria *E. coli* and enterococci as well as two groups of viruses (adenoviruses and enteroviruses) and the cytopathogenic effect were monitored in this pilot study. A number of chemical variables were also monitored to investigate whether some, like potassium, could be used to confirm that boreholes were indeed in the flow path of the groundwater contamination (since increases in at least potassium can be expected down-gradient of faecal pollution sources). Water levels were also determined.

Data assessment

Ogies sewage treatment works

- Potassium levels were found to be significantly different between the background borehole and each of the down-gradient boreholes. This suggested its possible usefulness for indicating flow path.
- *E. coli* was detected in three of the first four months (that may have been due to contamination during borehole construction) and thereafter not. This suggested that the containment borehole may be sufficiently far from the pollution source.
- No conclusive evidence was found that indicated that viruses travel further than *E. coli*.
- Enterococci were detected on a number of occasions when *E. coli* was not.
- Enterococci were detected in the background borehole in ten out of the twelve samples and *E. coli* twice. No good explanation for this was evident.
- A wide range of chemical constituents were found to have background concentrations significantly different from the down-gradient concentrations.
- No significant correlations were observed between microbial variables over time in the same borehole.
- No significant correlations were observed between each microbial variable at specified boreholes with a one month lag time. This suggests that monitoring as frequently as monthly will not cause serial correlation.
- No significant correlations were observed between the year's microbial dataset at one borehole (down-gradient of the pollution source) with the dataset at other down-gradient boreholes one, two and three months later.

Brits water care works

- As for Ogies, potassium levels were found to be significantly different between the background borehole and the source and middle down-gradient boreholes suggesting its possible usefulness for indicating flow path.
- Potassium levels were low in the containment borehole. Also, later estimates of groundwater flow direction from water levels indicated a direction significantly different from that estimated from the topography. The containment borehole was therefore regarded as not being in the flow path.
- As for Ogies, no conclusive evidence was found that indicated that viruses travel further than *E. coli*.
- As for Ogies, enterococci were detected on a number of occasions when *E. coli* was not.
- Some degree of microbial contamination in the background borehole was observed. However, this may have been caused by the surface contamination in the vicinity.
- As for Ogies, a wide, though different range of chemical constituents were found to have background concentrations significantly different from the down-gradient concentrations.

- As for Ogies, no significant serial or spatial correlations were found between microbial variables.

Potsdam sewage treatment works

- Although initial microbial contamination was detected in the containment borehole, the fact that it was detected only once thereafter suggests that the position of the containment, although not absolutely ideal, was probably as good a position as was possible under the circumstances.
- As for Ogies and Brits, no evidence was found that indicated that viruses travel further than *E. coli*.
- As for Ogies and Brits enterococci were detected when *E. coli* was not.
- As for Ogies and Brits, no significant serial or spatial correlations were found between the microbial variables.

Overall assessment

- No statistically significant evidence was present that suggested that there was contamination during construction of the borehole which then decreased thereafter.
- The conservative chemical constituents Cl, EC, K, Mg, and Na were found to be common to both Ogies and Brits as having significantly different background and down-gradient levels.

Refined procedures

Guidelines for geohydrologists for the placement of the containment borehole in particular have been improved on the basis of experienced gained in this pilot study.

Borehole construction specifications have been recorded that can be used as a basis of future drilling contracts.

Sampling procedures have been documented in some detail and include the use of photographs. Issues such as personal hygiene, personal health, the borehole sampling sequence (least contaminated first, more contaminated last), pump and pipe disinfection, borehole purging, actual sampling, sample preparation and sample transport have been addressed.

Inclusion of viruses

Inclusion of viruses in the national monitoring programme is likely to be essential if the stated objectives of the programme are to be achieved. This is primarily because of their different properties from bacteria (like *E. coil*) and the unpredictability of their behaviour. However, monitoring for viruses raises a number of unique problems.

Viruses require large sample volumes. One hundred litres is usually recommended although due to the inevitable associated problems, 20 litres is often used. A simple Monte Carlo model was developed to better understand why sample volume is such an issue. In essence, very low concentrations of viruses (*e.g.* 1 viral particle per 100 litres) are still associated with significant human health risks when used for drinking purposes. Using only 20 litres to "capture" a single particle in 100 litres obviously creates considerable potential for "false negative" results. (A false negative result reports that there are no viruses present when actually there are some present.) The model demonstrated the seriousness of this problem. Nevertheless, in accordance with current practice, 20 litre sample volumes are recommended. However, it is also recommended that an explicit statement be made regarding the likely decrease in information content in reports when zero viral particles are reported:

"These results are based on only 20 litre samples where at least 100 litre samples are theoretically more desirable. There is therefore a significant probability that results reported as zero viral particle/100 litres may be erroneous, i.e. low, yet problematic, numbers of viral particles may have been present but not detected. Firm conclusions cannot therefore be drawn directly about the extent to which such water is actually drinkable."

The problems associated with having to ensure samples (a) reach laboratories within 24 hours and (b) are kept cold were carefully considered. It was decided that these requirements cannot be relaxed.

Consideration was also given to which virus group would be the single group of choice for a national monitoring programme. Enteroviruses were recommended for a variety of reasons.

Analytical methods were also described and alternatives compared. Concentration of the viruses from the 20 litre sample can be achieved by ultrafiltration or using a glass wool trap. A comparison of recovery rates suggests that ultrafiltration is better than glass wool. However, careful consideration of when this is actually relevant revealed the following useful insight: If the detection of viruses is based on a presence/absence test, the difference between the recovery rates is only important when there are very few virus particles in the sample. It is proposed that the glass wool technique will be an acceptable concentration technique in the context of national monitoring. In this case, the loss of information content at low virus concentrations again necessitates an explicit statement to this effect in reports in which zero viral particles is reported. This is to ensure that water management decisions based on such data are suitably informed. The above statement can be modified as follows:

"These results are based on only 20 litre samples where at least 100 litre samples are theoretically more desirable. Furthermore, the virus concentration technique has inherently low virus recovery rates. There is therefore a significant probability that results reported as zero viral particles/100 litres may be erroneous (i.e. low, yet problematic, concentrations of viruses may have been present but not detected). Firm conclusions cannot therefore be drawn directly about the extent to which such water is actually drinkable."

In respect of the subsequent analytical technique, it is proposed that using an analytical technique that only detects presence or absence, like PCR, is satisfactory for viruses in the context of national monitoring.

The financial requirements relating to sampling and analysis were also considered. Various assumptions were made concerning:

- Transport costs (using couriers),
- Average distance travelled by couriers from towns near which samples were taken and the nearest laboratories,
- Analytical costs per sample (for bacteria and viruses),
- The number of participating laboratories,
- The number of monitoring boreholes, and
- The monitoring frequency.

These were used as the basis for a spreadsheet model which calculated the overall costs for sampling, transport and analyses for a series of scenarios. The results indicated the following:

If ultrafiltration is used to concentrate 20 litre samples in the laboratory:

- Based only on costs, a single laboratory is likely to be adequate for up to as many as 100 monitoring points (in respect of the number of simultaneous samples arriving at the laboratory and total number of samples per year).

- For 100 monitoring points, using five instead of one *E. coli* laboratory results in small savings (5%).
- A second virology laboratory (for example, in Cape Town) would probably only be justified when the number of samples per year increases to a few hundred.

It is also important to consider the disadvantages of only having one centralised laboratory when deciding on the number of laboratories required. These include a small pool of expertise, a possible inclination to inflate prices, and a lack of local inter-laboratory comparisons for quality control purposes.

If glass wool traps are used in the field to concentrate 20 litre samples:

- Costs are reduced to between 44% and 64% of those that would be incurred if ultrafiltration was used.
- In the initial years of implementation in which the number of monitoring points is less than 100, the analytical costs are considerably higher than the transport costs. (The opposite is true if ultrafiltration is used.)
- Not only are courier costs directly reduced but their use also considerably lessens the advantages of creating extra virology laboratories when the number of monitoring points increases significantly.

Human resource requirements as well as capital costs for equipment are also described in this report. It is noted that in the initial years of implementation in which up to about 100 samples per year are being analysed, the time required for both *E. coli* and viruses can probably be accommodated by existing laboratories. In other words, it is unlikely to be necessary to employ extra staff at those laboratories.

The following conclusions are drawn:

- As long as the physical logistics of getting samples from monitoring boreholes to the laboratory can be made routinely and confidently within 24 hours, then a single central laboratory is likely to suffice for some years.
- If in practice the courier companies are not able to guarantee the 24 hour delivery period, then another laboratory, *e.g.* in Cape Town may be necessary for monitoring boreholes in the west and south west of South Africa

It is recommended that monitoring of viruses (specifically the enteroviruses) be included in the national microbial monitoring programme for groundwater based on their high infectivity, their presence not being confidently indicated by a bacterial indicator such as *E. coil*, their having relatively small sizes and potentially long survival times under some conditions, and having a behaviour that is difficult to predict.

Conclusions

The following conclusions are drawn from this study:

- Microbial sampling of groundwater remains somewhat logistically and procedurally challenging, though procedures are now in place that should greatly reduce the risks of sample contamination on-site.

- Understanding the detailed behaviour of micro-organisms in the vicinity of a potentially significant faecal pollution source, like a sewage treatment works, remains challenging.

- Besides bacteria, as indicated at least by *E. coli*, viruses (specifically enteroviruses) are also infiltrating groundwater in the immediate vicinity of some sewage treatment plants.

- The use of *E. coli* as the indicator organism means that there is potential for false negative results (*i.e.* a zero result for *E. coli* indicating there is no health risk when there actually may be a health risk), at least in respect of enterococci bacteria as these were often observed when *E. coli* was not.

- There was no statistically significant evidence of initial microbial contamination (and subsequent decreases over time) of the boreholes during construction.

- Compared with the relative consistency over time of most chemical variables in groundwater in the immediate vicinity of sewage treatment works, the occurrence of bacteria and viruses is erratic and varies by orders of magnitude.

- Both the irregularity in the behaviour of both bacteria and viruses in groundwater and, in particular, the occasional appearance of viruses means that studies to better understand their dynamics in groundwater should be conducted over many years in order to obtain sufficient data for detailed statistical analyses. It is subjectively estimated that at least five years might be necessary at some sites.

- There is encouraging though not conclusive evidence that potassium can be used as an indicator to ensure that boreholes have been placed in the flow path down-gradient of a pollution source like a sewage treatment works.

- At some sites difficulties might occasionally be experienced in optimal placement of containment boreholes (*e.g.* because of close proximity to rivers or buildings). In these cases, placement nearer the pollution source, although not optimum, does not negate the proposed fundamental philosophy and design of the NMMP. It simply reduces the degree of useful monitoring information at these specific sites and places more emphasis on (a) improving the management of the pollution source and (b) monitoring at point-of-use boreholes down-gradient in the impacted aquifer.

- The very low infectious dose of viruses and their low concentrations in groundwater, theoretically necessitate very large sample volumes. This is notwithstanding the effects of processes such as adsorption and aggregation of viruses, which can worsen the problem. Although even 100 litres might be inadequate, the logistical problems of such large volumes necessitate a pragmatic choice of only 20 litres.

- In respect of recovery of viruses from large sample volumes (prior to their detection using PCR), the operating costs using the glass wool trap are likely to be significantly less than those associated with using ultrafiltration.

- As long as the physical logistics of getting samples from the monitoring borehole to the laboratory can be made routinely and confidently within 24 hours, then a single central laboratory is likely to suffice for many years. This applies to the use of ultrafiltration as well as glass wool, although much more confidently to the latter.

- The monitoring frequency can be as often as monthly without suffering from serial correlation.

- At the two fracture-rock aquifer sites (Ogies and Brits) five inorganic constituents were found to have significantly different concentrations between the background and down-gradient boreholes, namely Cl, EC, K, Mg and Na.