STUDIES ON MICROBIOLOGICAL DRINKING WATER QUALITY GUIDELINES

Report to the
WATER RESEARCH COMMISSION

by

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WRC Project No. 469/1/95
ISBN 1 86845 199 2

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EXECUTIVE SUMMARY

Background and Motivation

Water intended for human consumption should be safe, palatable and aesthetically pleasing. The methods used to determine whether water is safe vary according to guidelines and standards set throughout the world. Microbiological monitoring of drinking water has been practised in South Africa to various degrees for many years, but it has been argued that the introduction of microbial water quality standards is inapplicable at this point. A number of recommended guidelines and specifications are available and are used by central and local authorities at their discretion.

Rapid national and international changes in the approach to water quality, new technological advances and socio-economic conditions have emphasised the need to re-evaluate and reformulate microbiological drinking water quality guidelines. The available recommended guidelines lack suitable sampling statistics and do not describe the possible health implications of exceeding certain limits. Recently the Department of Water Affairs and Forestry has attempted to develop guidelines for various fitness for use including surface water to be used as drinking water. There is however a general misuse and confusion regarding certain terminology and the interpretation of microbiological guidelines.

Aim

The aims of this study were to conduct an in-depth investigation of available data on microbial guidelines and standards which are used in different parts of the world and to study the applicability of various guideline scenarios to the present situation in South Africa. International guidelines including the WHO, EEC, USA, Canadian, Australian, Japanese and Israeli guidelines were examined and compared to the specifications and recommended guidelines in South Africa.

Summary of results and conclusions

The objectives of monitoring microbial water quality are to ensure the protection of public health. The issues surrounding monitoring of microbial water quality are vast and in this study they have been addressed in three major categories, namely, microbiological, water quality and sampling issues.

Microbiological issues include the justification of the utilization of indicator organisms, which indicator organism to test for, collection and preservation of samples, examination procedures and confirmation steps. The question of the utilization of specific indicators in water quality guidelines is the major issue to be addressed in formulating water quality guidelines. Choosing an indicator is directly related to the objective set for water quality monitoring. As microbial drinking water quality guidelines aim at ensuring both the protection of human health and the evaluation of the treatment efficacy more than one indicator organism is often

needed. The coliform group of bacteria has been used more than any other indicator group for monitoring drinking water because it addresses both health and treatment efficacy objectives. Although the total coliform group can only remotely indicate human health risk, the group includes faecal coliforms and more specifically, *E. coli* which originates from faecal matter. Secondary indicators (heterotrophic plate counts, faecal streptococci, *Clostridium, Pseudomonas aeruginosa* and *Aeromonas*) are generally not included in guidelines, but are commonly used for supporting data. Because the potential presence of pathogens in water cannot be predicted solely by faecal indicators it may, under certain circumstances, be necessary to monitor for the presence of pathogens. In general it is recommended that water intended for human consumption should not contain any pathogens, which include protozoan parasites, enteric viruses and pathogenic bacteria such as *Salmonella, Shigella, Vibrio cholera, Yersinia* and *Campylobacter*.

Water quality issues dealt with in this study include the significance of the presence of indicators, statistical parameters for indicator presence, protection of public health in relation to indicator levels, steps required following unsatisfactory samples and interpretation of results. The significance of the presence of indicator organisms is inconclusive as inadequate information is available regarding the margin of safety with respect to any coliform count with which to set a standard. Research does not support a quantitative relationship between coliform density and pathogen density and the potential for outbreak of water-borne diseases. Most guidelines are based on water quality considerations with little direct relation to human health. However, it is still recognised that coliforms are the best available indicators of microbial quality.

Sampling issues were addressed and include the time period for water quality evaluation, sample number and frequency, site selection, sampling time and additional samples. Initially sampling numbers and frequency were not specified as guidelines were expressed as absolute levels of safety. Presently though, this approach has altered in most guidelines. The basic aim of a sampling programme is to ensure that the quality of the water sampled represents the quality of the water supply. It is necessary to ensure that the sampling programme will detect contamination, if it occurs. The majority of guidelines and standards recommend that the frquency of sampling be dependent on the size of the population served. This principle is based on the assumption that as the population size increases, so will the size and complexity of the system and thus the chances of contamination. The fraction of samples which is permitted to be positive is also specified (usually 5% of samples in a month or year). The use of the principle of frequency of sampling based on population served is empirical and devoid of any mathematical basis. Examination of larger samples (200 - 1000mℓ) has been suggested and would be statistically more meaningful and reduce the risk of failure to detect low levels of coliforms. Sites for sampling need to be chosen with care to allow for samples which represent a large area covered by a distribution system. It has been recommended that the best approach for the selection of sampling locations for monitoring the microbial water quality in the distribution system is stratified random sampling, but this approach is rarely practised due to practical problems.

The most lax of all international guidelines and standards are those of the WHO which have been devised to accomodate 3rd World countries. The WHO states that the adoption of too stringent drinking water quality standards could limit the availability of water supplies that meet those standards. This is a significant consideration in regions of water shortage, such as South Africa. Guidelines should not only address high quality purified potable water, but also be appropriate for areas in which only localised purification schemes and limited infrastructures for water supplies are available. However, only water supplied to the consumer should be evaluated as drinking water. To develop guidelines for communities where no water supplies are available and surface waters are used will require an holistic approach to surface water management in which pollution sources are well managed.

The latest guidelines published for South Africa are those of the DWA&F (1993). Of the South African guidelines, the SABS specifications is the most commonly used by water authorities and municipalities, even though the three tiered system of Aucamp and Vivier (1990) has been accepted in principle by the Department of National Health. With the latest release of the DWA&F (1993) guidelines for domestic water it is even more important that an official joint South African guideline for microbial drinking water quality is accepted on a national level. In a developed country the objectives of monitoring water microbial quality are to ensure protection of public health and at the same time to evaluate the efficacy of the water-treatment processes. The SABS specifications address the protection of human health under the assumption that such treatment purification process is provided. This will be suitable in urban areas where conventional purification processes are available. These guidelines are based solely on limits for indicator organisms which will facilitate water of high quality as long as conventional treatment processes are functioning well. However many parts of South Africa do not fit this description which limits the applicability of these guidelines.

The WHO (1984) states that the adoption of too stringent drinking water standards could limit the availability of water supplies that meet those standards. This is a significant consideration in South Africa. It will therefore not be suitable for South Africa to adopt the "Coliform Rule" of the USA which requires monitoring based on the presence or absence of coliforms in a sample. Even though this rule does not require quantifying the coliform density, and thus appears to be a more simple rule, it has been calculated that the rule is twenty times more stringent than the rule based on maximum contaminant levels, and is thus not suitable for the South African situation. Since South African guidelines should cater to all populations of South Africa, one has to take the same approach as the WHO in balancing water quality and availability.

South African guidelines should be revised, particularly with regards to the inclusion of limits for pathogens and the latest methodologies for the detection of both indicator organisms and pathogens such as enteric viruses and protozoan parasites. The DWA&F guidelines have included coliphages as indicators of enteric viruses, but mention the limitations on their usefulness as viral indicators as well as advising that the guideline should be considered as extremely tentative. It is

believed that the present indicator organisms recommended in the SABS specifications are adequate for the routine monitoring of drinking water, but if problems are suspected then additional tests should be specified, for example, testing for the presence of *Vibrio cholerae* and *Salmonella spp.*, in areas where non-point source pollution occurs and examination of water for *Aeromonas spp.* and *Pseudomonas*, depending on the type of water and distribution system. It is also believed that water should be examined for the presence of enteric viruses and protozoan parasites, such as *Giardia* and *Cryptosporidium* on a routine but less frequent basis (*eg.* monthly).

Meaningful statistical descriptions of data processing needs to be addressed in South African guidelines. Statistical aspects of water quality monitoring, for instance the monitoring frequency and data analysis, ie. central tendency and variability. The monitoring frequency should be re-examined in light of data available demonstrating that there is very little benefit from a statistical point of view for taking more than 150 samples/month and fewer than 50 samples/month. The principle of frequency of sampling based on population size has been criticised because it lacks any mathematical basis.

Recommendations for further research

It will be necessary to establish a task group for the assessment and re-evaluation of drinking water quality guidelines for South Africa. The task group should include members of governmental departments concerned with water quality and health, organisations actively involved in microbiological aspects of drinking water quality, as well as major water boards and authorities. In addition to re-assessing the guidelines the task group should also examine the feasibility of establishing drinking water *standards* for microbiological quality of drinking water in South Africa.

1. BACKGROUND

Microbiological monitoring of drinking water has been practised in South Africa to various degrees for many years. At present a number of suggested or recommended guidelines and specifications are available, and are used by central and local authorities and other regulatory bodies concerned at their discretion. The SABS specification were reformulated in 1984 and although interim evaluation of microbiological methods has taken place, no re-evaluation of the current specifications and recommendations and their implications has been carried out by any central committee for a number of years. Recently the Department of Water Affairs and Forestry (1993) has developed guidelines for various fitness for use including surface water to be used as drinking water. The DWA&F document is not intended as a guideline document *per se,* but as a document to be used for the development of limits for managing water resources for particular areas and uses. However there is a general misuse and confusion in the country regarding certain terminology and the interpretation of microbiological guidelines.

Rapid national and international changes in the approach to water quality, new technological advances and changes in socio-economic conditions create the need to re-evaluate and, if necessary revise microbial guidelines for drinking water quality. The available recommended guidelines lack proper sampling statistics, and do not describe the possible health implication of exceeding certain limits. The recent growth in unstructured urbanisation in South Africa and the increased threat of pollution to drinking water sources emphasise the importance of re-evaluating and reformulating the current guidelines.

The aims of this study were to conduct an in-depth investigation of available data on microbial guidelines and standards which are utilised in different parts of the world and to study the applicability of various guideline scenarios to the present situation in South Africa. This study focuses on drinking water quality as the final product presented to the consumer, *ie.* after treatment, storage and distribution.

2. INTRODUCTION

It is estimated that worldwide 80% of all disease is attributable to inadequate water and sanitation and at any time one half the hospital beds in the world are occupied by people with water-related disease (Bourne, 1982). According to WHO estimates in 1985, the number of people suffering from the principal water-related diseases at any one time is about 1,25 billion (Lewis, 1985).

According to the majority of international guidelines and standards, water intended for human consumption should be safe, palatable and aesthetically pleasing. This therefore implies that the water used for drinking purposes should ideally be free of pathogenic microorganisms and other substances that may present a health risk. The methods used to determine whether water is safe, palatable and aesthetically pleasing vary according to guidelines and standards set throughout the world. In

South Africa, the Department of Water Affairs and Forestry is responsible for the administration of the "Water Act" of 1956 and the Department of National Health and Population Development exerts its authority to ensure that the risk of contamination of water is eliminated (Anon, 1990).

The objectives of monitoring water microbial quality are to ensure protection of public health and at the same time to evaluate the efficacy of the water-treatment processes. Although these objectives are related, they require different monitoring approaches, thus complicating the formulation of microbial water quality guidelines.

Most guidelines available are based on water quality considerations with little direct relation to human health. Epidemiological studies are of value in establishing water quality criteria based on the relationship between levels of water quality indicators and the health risk associated with the use of different water types. Epidemiological studies also maintain current awareness of the possibility of outbreaks and new aetiological agents (Craun, 1978). Only a few guidelines have been based on epidemiological considerations which mostly address recreational water quality.

The terms used to assess microbial water quality (standards, objectives, criteria and guidelines) differ throughout the world. The term "standard" applies to any definite rule established by authority and is legally enforceable. The process of setting standards is slow, complex, imperfect and based on difficult qualitative judgements (Boyd *et al.*, 1986). "Objective" or "guideline" represents an aim or goal, and "criterion" designates a condition defined by means of a critical review on scientific information. It carries no connotation of authority, nor does it imply an ideal condition (Chiaudanis and Premazzi, 1988; Aucamp and Vivier, 1990; Mc Neill, 1985).

The issues surrounding monitoring of microbial water quality are vast and in this study have been addressed in three major categories *ie.* microbial, water quality and sampling issues.

3. MICROBIOLOGICAL ISSUES

3.1. Indicator Organisms

3.1.1. *Justification for the utilization of indicator organisms*

Ideally drinking water should not contain any known pathogenic microorganisms and it should be free from bacteria indicative of pollution with excreta. To ensure that a supply of drinking water satisfies these guidelines of bacterial quality it is important that water be examined regularly for indicators of pollution (WHO, 1984). Routinely it is impossible to test the water supply for all pathogens related to water-borne diseases because of the complexity of the testing and the time and cost related to it. It is preferable to use indicator systems which are able to index the presence of pathogens and related health risks in water.

Because it is preferable to use indicator systems, ideally an indicator organism should fulfil a number of criteria:

- it should be present when the pathogen is present and should be absent in unpolluted water;
- it should be present in numbers greater than the pathogens it indicates;
- its survival in the environment and resistance to treatment processes should be comparable to that of pathogens
- it should not be harmful to human health
- it should be easy to identify and isolate (Berg, 1978).

At present, there is no absolute indicator which complies with all the above criteria, but the traditional indicators of drinking water quality include the coliform group (including *E. coli*), faecal streptococci, and *Clostridium perfringens* (a sulphite-reducing, spore forming anaerobe). More recently the faecal coliforms, or thermotolerant coliforms and *E. coli* have been differentiated from the total coliforms as more specific indicators of faecal pollution (Mc Neill, 1985). The standard plate count is also used in many countries, including South Africa, as a useful parameter in the quality control of water and water treatment processes.

3.1.2 *Characteristics of major indicator organism groups:*

3.1.2.1 Total Coliforms:

The coliform group is defined as any Gram negative, oxidase negative, non-sporing, rod shaped organisms capable of growth in the presence of bile salts and capable of fermenting lactose with the production of acid, gas and aldehyde within 48 hours (WHO, 1982). Total coliforms include the genera *Escherichia, Citrobacter, Klebsiella* and *Enterobacter*. Coliform bacteria should not be detected in treated water supplies, and if found, suggest inadequate treatment or post-treatment contamination.

3.1.2.2 Faecal Coliforms:

Faecal (thermotolerant) coliforms refer to those coliforms which retain the specified fermentative properties at 44.5°C and include the genera *Escherichia* and to a lesser extent occasional strains of *Enterobacter, Citrobacter* and *Klebsiella* (WHO, 1984). Of these organisms, only *E. coli* is specifically of faecal origin, being found in the faeces of man, animals and birds in large numbers. Presumptive *E. coli* are defined by the WHO (1984) as those faecal coliforms that ferment lactose and mannitol with acid and gas production at 44 or 45°C and which also produce indole from tryptophan.

3.1.2.3 Faecal Streptococci

The occurrence of faecal streptococci in water is a general indication that faecal pollution has occurred. Although present in large numbers in faeces they are considerably less numerous than the coliform group in human faeces. The term faecal streptococci refers to streptococci which are coagulase negative and which

are capable of growth at 44.5°C in the presence of 40% bile and in concentrations of 0.04% sodium azide, which are normally inhibitory to most Gram negative bacteria including coliforms. Faecal streptococci are normally present in the faeces of humans and other warm-blooded animals and are therefore generally considered to be suitable indicators of faecal pollution. Faecal streptococci include species which belong to the Lancefield's serological groups D and Q (APHA, 1980). The enterococcus group of faecal streptococci includes *S. faecium* and its variants, and *S. faecalis* and its subspecies. *S. avium* belongs to the group Q streptococci and accounts for some of the organisms previously designated as biovars of group D streptococci (Mc Neill, 1985).

3.1.2.4 Clostridia

Clostridia are anaerobic, spore-forming, sulphite reducing organisms generally regarded as indicators of faecal pollution, occurring in the faeces of man and other animals. Their spores are resistant to environmental stress and may therefore be used to detect remote sources of pollution (WHO, 1984; Mc Neill, 1985). The spores are also highly resistant chlorine.

3.1.2.5 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa occurs in the faeces of man and rarely in the faeces of lower animals (Wheater, 1979). In drinking water, the presence of *Pseudomonas aeruginosa* may relate to biofilm formation and may occur in the absence of coliform organisms (Reitler and Seligmann, 1957). The biofilm may provide a protective environment for *Pseudomonas* resulting in high chlorine resistance. *Pseudomonas aeruginosa* is both an indicator organism and an opportunistic pathogen invading individuals in a debilitated state, being implicated in eye and ear infections, therefore its detection has a direct health implication.

3.1.2.6 Standard plate counts

Standard plate counts (SPC) represent those microorganisms present in water which are able to form colonies in nutrient media under specified culture conditions, *ie.* 37°C within 48 hours or 22°C within 72 hours, (WHO, 1984; Mc Neill, 1985).

3.1.2.7 *Aeromonas*

Aeromonas species are present in high densities in sewage, but because they are ubiquitous in nature their value as an indicator of faecal pollution has been questioned. *Aeromonas* may be valuable as an indicator indexing the general deterioration of nutrient enriched waters (Mc Neill, 1985). *Aeromonas* species have been isolated from both chlorinated and non-chlorinated water supplies in Australia. With the chlorinated water supply, the incidence of *Aeromonas* gastroenteritis paralleled the pattern of isolation of *Aeromonas spp.* in water in the distribution system (Watkins and Cameron, 1991).

3.1.2.8 Bacteriophages

Phages have been proposed as alternative indicators of enteric viruses in contaminated waters because they are present in numbers greater than or equal to enteric viruses, are more resistant to disinfection than enteric viruses, persist in water longer than enteric viruses and are detected using simple, rapid and economical techniques. However many limitations exist. Phages have also been proposed as indicators of faecal pollution (Kott, 1984, Borrego, *et al,* 1987, Morinigo, *et al*, 1992,)

3.1.3 *The utilization of specific indicators in water quality guidelines*

This question is the major issue to be addressed in formulating water quality guidelines. Choosing an indicator organism is directly related to the objective set for water quality monitoring. As microbial drinking water quality guidelines aim at ensuring both the protection of human health and the evaluation of the treatment efficacy more than one indicator organism is often needed. Some of the indicators specifically address treatment efficacy with no, or very little, emphasis on human health.

3.1.3.1. Coliforms

The coliform group of bacteria has been used much more than any other indicator group for monitoring drinking water because it addresses both health and treatment efficacy objectives. To date, the coliform group is still the most reliable indicator for drinking water. Although the total coliform group can only remotely indicate human health risk the group includes *E. coli,* which usually originates from faecal matter. The total coliform group was used in the US since 1914 under the US Treasury Department Standards. Total coliforms continue to be used to date in the US for regulating drinking water quality, but with additional tests for presence of faecal coliforms or *E. coli* (AWWA, 1990). Other drinking water microbial standards and guidelines which specify total coliforms include Australia criteria, WHO guidelines (Europe and International), EEC directive, Canadian guidelines, Israeli standards and the South African SABS specifications and guidelines proposed by the Department of Health and Population Development (Table 1). The major deficiencies of the coliform group are their natural survival pattern which differ greatly from known bacterial pathogens coinciding with their ability to regrow in water distribution systems. Other deficiencies include their different response to conventional water treatment processes than enteric pathogens and the suppression of their growth by high populations of other microorganisms. Many countries continue using coliforms as indicators of drinking water quality to allow for comparison with historical data.

3.1.3.2. Faecal Coliforms

In the late 1940's the total coliform group was split to include a subgroup, namely the faecal coliform group. This group is more representative of faecal pollution and thereby be more indicative of possible health implications. It should be recognized that the faecal coliform determination does not distinguish between human and animal faecal contamination. Generally no faecal coliforms should be present in drinking water. Most recent standards, guidelines and criteria include this indicator subgroup of faecal coliforms, however only in some of these guidelines a direct analysis of the subgroup is specified (Table 1). The term *faecal coliform* is often confused with the species *E. coli*. The faecal coliform group includes other species of bacteria besides *E. coli* and not all are of faecal origin.

3.1.3.3. Faecal Streptococci

Faecal streptococci are not included in guidelines and are commonly used as secondary indicators *ie.* for supporting data only. The faecal streptococci group includes species that may not necessarily be of faecal origin. Taxonomic problems determining the origin of faecal streptococci isolated from soil and vegetation have been reported (Kibbey *et al.*, 1978; De Wet *et al.*, 1991). The confusion of strains of streptococci originating from plants with those from faecal origin may mislead the interpretation of the significance of faecal streptococci in waters receiving surface run-off. Unless strain identification is part of the routine procedure, it is not advisable to use faecal streptococci as the only indicator for monitoring drinking water quality. Faecal streptococci may be used as a secondary indicator organism, for example, if there is a doubt about the nature of contamination, especially when coliform organisms are found in the absence of faecal coliforms and *E. coli* (WHO, 1984).

3.1.3.4. Clostridia

The sulphite-reducing clostridia may also be used as secondary indicator organisms and may be used to indicate deficiencies in water treatment processes and were therefore included in previous guidelines (Aucamp and Vivier, 1990). However the spores appear to be too resistant to chlorination to be used as an indicator of drinking water quality. The cultural methods available for clostridia are difficult to apply on a routine basis. In general it is not recommended to include this group of organisms in the routine monitoring of distribution systems as they are capable of surviving and accumulating in the distribution system, thereby giving rise to false alarms (WHO, 1984; Mc Neill, 1985).

3.1.3.5. *Pseudomonas aeruginosa*

Pseudomonas aeruginosa has been recommended for assessing the hygienic quality of drinking water as it occurs in the faeces of man and rarely in the faeces of lower animals (Wheater, 1979). In drinking water, *Pseudomonas aeruginosa* may occur in the absence of coliform organisms (Reitler and Seligmann, 1957). The WHO (1984) have recommended that the detection of this organism should not be used

for the routine monitoring of water for the presence of faecal pollution. Its importance lies in its suitability as a water quality indicator of swimming pools (Mc Neill, 1985).

3.1.3.6. Standard Plate Count (SPC)

In 1985 three alternative methods for determining the total number of bacteria (heterotrophic plate count - HPC) in a water sample were approved by the American Standard Methods Committee, namely the pour plate, spread plate and membrane filtration methods (Standard Methods, 1989). Both the SPC and HPC are not able to indicate faecal pollution, but are useful in assessing the efficiency of water treatment processes and in the interpretation of coliform counts, since total bacterial densities exceeding 500 colony forming units (cfu)/ml may suppress the growth of coliforms resulting in false negatives. Therefore if the heterotrophic plate count exceeds 500 cfu/ml then the sample is considered to be coliform positive even if no total coliforms were detected (Geldreich *et al.* 1978, De Zuane, 1990).

3.1.3.7. *Aeromonas*

Water undertakings in the Netherlands have adopted tentative guidelines for *Aeromonas spp., ie.* less than 20/100ml as a 90 percentile within one month for final drinking water (Hayes, 1989).

3.1.4 *Exceptions where pathogen presence is set in water quality guidelines*

Because the potential presence of pathogens in water cannot be predicted solely by faecal indicators it may be necessary under certain circumstances to monitor for the presence of pathogens in addition to routine indicators provided that the facilities are available. The WHO (1984) have recommended that under certain circumstances it is necessary to monitor for *Salmonella spp., Shigella spp., Vibrio cholera, Yersinia enterocolitica, Campylobacter fetus,* enteropathogenic *E. coli and* enteric viruses, whereas in Australia it has been recommended to monitor for *Salmonella sp., Vibrio cholerae, Shigella spp., Yersinia, Leptospira, Legionella, Giardia, Naegleria fowleri,* enteric viruses, nematodes, cestodes and trematodes (Mc Neill, 1985). The EEC Directive (1980) specifies that water intended for human consumption should not contain pathogens and if it is intended to supplement the microbiological analysis of water intended for human consumption, the samples should be examined for pathogens including *Salmonella,* pathogenic staphylococci, enteroviruses and faecal bacteriophage.

3.1.4.1. Protozoan parasites

Parasites, as do viruses, have a low infective dose and a greater resistance to chlorine than faecal indicators. Both *Giardia* and *Cryptosporidium* have caused water-borne outbreaks throughout the world. Methods have been developed for the detection of these organisms in water. In general no cysts or oocysts should be present in water. In the USA, the EPA (1989) requires that a reduction in the

treatment process should ensure a 3 log reduction of *Giardia* cysts. Most guidelines merely state that pathogens should be absent from drinking water. The DWA&F (1993) have included suggested guidelines for protozoan parasites for the first time. It is suggested that water to be used directly or to receive treatment should contain <1 *Giardia* cyst/10ℓ and <1 *Cryptosporidium* oocyst/10ℓ.

3.1.4.2. Enteroviruses

There is general agreement that human viruses are not acceptable in drinking water and that virological analysis may be necessary under certain circumstances. Viruses have been isolated from drinking water supplies in the absence of faecal indicators and with 0,8mg/l of free chlorine (Watkins and Cameron, 1991). Because bacterial indicators are not able to indicate the potential viral health risks associated with water, direct tests for viruses are recommended for certain situations where virological facilities are available (WHO, 1984; EEC, 1980; Slade, 1985 and Mc Neill, 1985). Suggestions as to when virological testing should be carried out include the following: raw water evaluation to give an indication of any health hazard should treatment fail; to check on an installed treatment process to demonstrate virus removal; on any occasion when treatment is deficient; service reservoirs and parts of distribution systems where contamination is suspected; periodic checks on underground sources and during outbreaks of possible water-borne viral disease (Slade, 1985). It has often been suggested that coliphages are suitable indicators of the presence and behaviour of viruses as they are present in faecal wastes and therefore serve as indicators of pathogenic viruses (Grabow, 1991; IAWPRC Study Group, 1991). However, limitations in the use of coliphages as indicators of enteric viruses include the inability of coliphages to indicate the presence of viruses and a lack of correlation between densities of coliphages and enteroviruses in raw sewage (McNeill, 1985).

3.2. Preservation and Collection of Samples

The basic aim of collecting samples is to obtain a sample representative of the quality of the water body supply. The samples must be collected so that the analytical results represent the water quality and its spacial and temporal variability during the time period of interest (WHO, 1984). Correctly handled collection of water samples is crucial for obtaining valid microbiological results. Most international guidelines and standards provide procedures for the collection of samples and most guidelines and specifications recommend sampling procedures (WHO, 1984; SABS, 1984; APHA, 1980; US-EPA 1986; ISO 1980, 1982; Standard Methods, 1989). The EEC directive (1980) does not supply guidelines on sample collection, storage or analysis. The SABS specifications (1984) include the procedures for sampling water from taps, boreholes and wells for drinking water. This includes general hygiene principals such as washing hands before sampling, using sterile sampling bottles and ensuring that no surface of the closure be allowed to touch the hand or any other object. The sample bottles should be at least 250ml, clean and sterile.

Neutralization of disinfectants is necessary if the water to be examined is likely to contain chlorine, chloramine, chlorine dioxide or ozone, so that the sample analysed indicates more accurately the true microbial content at the time of sampling. This is achieved by the addition of 0,1mℓ of an 18g/litre solution of sodium thiosulphate per 100ml of bottle capacity (WHO, 1984). This concentration is capable of neutralizing at least 5mg of available chlorine per litre and should be suitable for routine sampling. This concentration of thiosulphate has no significant effect on coliform organisms, including *E. coli* during storage.

When a number of samples are to be taken for various purposes from the same location the sample for bacteriological examination should be collected first to avoid the danger of contamination of the sampling point. When collecting samples directly from a reservoir, spring, shallow well, stream, river or lake samples must not be taken too near the bank or too far from the point of draw-off and areas of stagnation must be avoided (WHO 1984). The sample should be taken with the mouth of the bottle facing the current or if no current exists, the bottle should be held horizontally through the water. When sampling from distribution systems, the taps chosen must be supplied with water direct from the public main and the water should be let to run to waste for several minutes to ensure that the water in the pipe work is flushed out before the sample is taken.

The DHSS (1969), Australian Standard (1981) and WHO (1984) recommend that storage of samples should be kept to a minimum time period of 24 hours, provided that the samples have been kept cool (preferably between 4 and 10°C, but not frozen) and in the dark. If delays are unavoidable samples may be filtered on site and transferred to the laboratory in transport media in airtight containers before transfer to conventional media for final examination in the normal way. The SABS specifications state that microbiological analyses must be carried out within 6 hours of sampling. If a sample is between 6 and 24 hours old the sample may be analysed, but the examination does not comply with the requirements of the specification and such results may only be used for purposes of information.

3.3. Examination Procedures for the Measurement of Indicator Density

Two basic procedures are used for the enumeration and detection of the major indicator organisms, *ie.* total and faecal coliforms. These procedures include the membrane filtration (MF) method and the multiple tube method, also known as the most probable number (MPN) method. Methods should yield results rapidly to allow for speedy remedial action during water pollution events. Mc Neill (1985) discussed the procedures and their advantages and limitations which have been summarised as follows. The membrane filtration method is carried out by the filtration of a measured volume of sample through a membrane filter of 0.45nm pore size, which retains bacteria on the filter surface. The filters are incubated on a selective medium and the colonies that develop represent a direct count of the number of bacteria in the original sample. The MPN method involves the inoculation of decimal dilutions of a sample into replicate tubes of a selective liquid medium. The most probable number of indicator organisms are estimated with the aid of probability

tables.

The advantages of the membrane filtration method include rapid results, precision, ability to process large volumes, cost and time savings, smaller confidence intervals than for the MPN method and additional information on changes in water quality being detected by assessing the non-indicator flora. The limitations of the membrane filtration method include clogging of filters by turbid samples, masking of indicators by presence of high numbers of general bacterial population and poor recovery of stressed indicators. The advantages of the MPN method are that turbid samples may be analysed, toxic substances are diluted and the method is suitable for sediments. The limitations of the MPN method include lack of precision, long time period required for confirmation of tests, large volumes cannot be analysed, the method is labour intensive and costly and it is not applicable for field work.

Most guidelines or standards allow either of the two methods to be used, although the membrane filtration method is generally the preferred method, except for turbid samples. A large variety of media are used throughout the world for the enumeration and detection of indicator organisms, thereby making comparisons between laboratories very difficult, if not impossible. The 1984 South African specifications only recommended the membrane filtration method for the examination of water, but the more recent SABS specifications (SABS methods, 1990) now recommends both membrane filtration and the most probable number method. For the membrane filtration method m-Endo agar LES and m-FC agar are specified for the enumeration of total and faecal coliforms respectively. The MPN method specifies the use of lauryl tryptose broth and brilliant green bile broth for the enumeration of total and faecal coliforms respectively.

The MPN method has a very large sampling error in comparison to the MF method. The MPN method may also have many false positive reactions which are eliminated in the MF method (DHSS, 1969).
Counts from the MF method also are subject to statistical variation and replicate counts will not in general show the same number of organisms. The confidence limits for the true number of organisms can be calculated as (DHSS, 1969):

upper limit = $C + 2 \times (2 + \sqrt{C})$ where C = counts
lower limit = $C - 2 \times (1 + \sqrt{C})$

eg. if 100 counts are observed then the true number could lie between 78 and 124.

In the USA the Total Coliform Rule is based on the presence-absence test and is now the mandatory test for the examination of drinking water (AWWA, 1990). Because the traditional MF and MPN methods had disadvantages the new system was developed that allows for easy interpretation within 24 hours (Olson *et al.*, 1991). All positive samples need to be examined to establish whether faecal coliforms are present. If a coliform positive sample is also faecal coliform (or *E. coli*) positive then the water system is in violation of the maximum contaminant level (AWWA, 1990).

The methods recommended for standard plate counts are generally based on the pour plate technique which involves the direct introduction of the water sample to the culture media. However, the media, incubation temperature and times recommended differ from one guideline to another.

3.4. Confirmation Steps

Should any coliform organisms be present in a finished water it is important that confirmation and differentiation be taken as far as possible to determine whether the contamination is faecal in origin and to aid in tracing the source.

The SABS Methods - 221 (1990) recommend that any presumptive total or faecal coliforms isolated using the membrane filtration method be subcultured into lactose peptone broth and incubated at 37°C or 44.5°C for 48h or 24h respectively and examined for gas production. To confirm that *E. coli* is present it is recommended that a confirmed faecal coliform is subcultured from the lactose peptone broth into tryptone water and tested for the production of indole after 24h incubation at 44.5°C. If the most probable number method has been used it is recommended that positive total and faecal coliforms be subcultures into brilliant green bile broth and examined for gas production after incubation at 37°C or 44,5°C for 48h or 24h respectively.

Confirmation steps recommended in the WHO guidelines (1984) and Standard Methods (1989) both mention similar techniques to those described in the SABS Methods, but additional methods are included. In addition to the methods described, EC broth may also be used in the confirmation of faecal coliforms. Rapid verification is also recommended by testing for cytochrome oxidase (coliforms are cytochrome oxidase negative) and ß galactosidase, where results may be obtained within 4 hours. Commercially available kits are also recommended in Standard Methods (1989).

In June 1990 three methods for testing for *E. coli* were proposed in the USA *in lieu* of faecal coliforms, based on the ability of *E. coli* to produce the enzyme beta-glucuronide, which hydrolyses the 4-methyl-umbelliferyl-beta-D-glucuronide (MUG) contained in the selective media to form 4-methyl-umbelliferane, which fluoresces when exposed to UV light (Pontius, 1992). Two of the 3 methods were approved in January 1991, namely EC medium plus MUG and nutrient agar plus MUG (Pontius, 1992). Olson *et al.* (1991) compared the membrane filtration method with two commercially available MUG methods for the detection of total coliforms and found that the membrane filtration method was superior to one of the commercial tests (Colilert) and equivalent to the other (Coliquik), if atypical colonies were taken into account as recommended in Standard Methods for the detection of total coliforms. Clark *et al.* (1991) found that the agreement between the MF method and the Colilert and Coliquik tests for *E. coli* detection was relatively poor in water samples collected from a fully treated drinking water reservoir. Higher levels of agreement were observed from untreated source water samples. They recommended that the use of the MUG methods for the detection of *E. coli* should

incorporate a confirmatory step that ensures that negative samples are indeed true negatives and not false negatives.

McFeters *et al.,* (1993) recently found the new enzyme detection methods for the detection of low levels of coliforms and *E. coli* (Colilert, Colisure and Coliquik) in both source water and treated water to be sensitive and more rapid than the standard methods.

4. WATER QUALITY ISSUES

4.1. Significance of the Presence of Indicators

Although many pathogens may be detected in water, the methods of detection and enumeration are often time consuming, complex and expensive. Because it is essential that water is monitored frequently and regularly it is important that simple tests are used instead of infrequently by complex tests (WHO, 1984). The approach to monitoring the quality of water is therefore to detect organisms normally present in the faeces of man and warm blooded animals as indicators of faecal pollution. The presence of such organisms indicates the presence of faecal material and therefore intestinal pathogens may also be present. Conversely, the absence of faecal commensal organisms indicates that pathogens are most likely also absent (WHO,1984). The use of classical faecal indicators presupposes that faecal wastes are the exclusive source of pollution, that all water-related infections are enteric, thereby excluding those water-contact and water transmitted infections associated with non-faecal pathogens (Mc Neill, 1985). There are many limitations of the traditional indicator system which include the following:

* the frequency of a faecal indicator may not accurately estimate the health hazards associated with the presence of opportunistic pathogens which may multiply in a nutrient enriched environment

* the health risks associated with sewage receiving waters may be over estimated if an indicator is capable of multiplying in sewage

* indicator to pathogen ratios may be lower under epidemic situations

* there may be a wide range in indicator to pathogen ratios coincident with decreases in the number of contributing individuals

Bacterial indicators are not able to indicate the potential viral health risks associated with water, therefore direct tests for viruses are necessary under certain circumstances, such as, determining the efficiency of treatment processes and wastewater re-use (Mc Neill, 1985). It has been recommended by a WHO Scientific Group (1979) that drinking water, raw water sources, wastewater effluents, marine recreational waters and shell fish growing waters subject to wastewater and sludge contamination be examined for the presence of human enteric viruses, but

according to the WHO (1984) guidelines the most realistic approach for controlling the transmission of viruses through drinking water is to recommend consistently meeting the treatment criteria that have been found to be effective in preventing water-borne viral disease. The only practical and economical approach to monitoring the microbiological safety of drinking water is the frequent examination for the occurrence of faecal indicator bacteria.

There is general agreement that the desirable limit for total coliforms should be zero, but according to a discussion at an AWWA workshop in 1985 (Tobin, 1988) there is inadequate information about the margin of safety with respect to any coliform count with which to set a standard. The new Coliform Rule of the USA which was finalized and announced in 1989 requires a monitoring programme based on the presence or absence of coliforms in a sample. The implications of results obtained from these tests differ from those estimating densities of coliforms. One of the major reasons for this change in strategy is that research does not support a quantitative relationship between coliform density and pathogen density and the potential for outbreak of water-borne diseases (Borup, 1992). It is still recognised that coliforms are the best available indicators of microbial quality. The AWWA Technical Advisory Work Group calculated that the new total coliform rule is twenty times more stringent than the previous rule based on maximum contaminant levels.

Giardia and *Cryptosporidium* have been isolated from raw and treated drinking water on isolated occasions (Kfir *et al,* 1993). Because the infective dose of these parasites is extremely low it is of significance to public health. These parasites are extremely resistant to treatment processes and are minimally affected by chlorination. According to the WHO (1984) *Giardia* is the most commonly identified aetiological agent of water-borne disease outbreaks. Most outbreaks have been associated with treatment breakdowns or partial treatment.

Making decisions regarding the acceptability of water would be easy if the overall effect in the water quality deviation could be expressed in an integrated manner, giving due regard to both the importance of each constituent as well as the magnitude of its exceeded concentration (Bhargave, 1985). Upper and lower levels can be set for drinking water by an "index" which represents the overall integrated effect of all the water quality variables and their respective concentrations as well as the related implications of drinking such water. An index is essentially a fraction with the quantity to be measured being the denominator and the standard being the numerator (Inhaber, 1976). Bhargave (1985) describes how the index can be evaluated so that the effect of variables with different importance are appropriately taken care of. For example, if coliform levels rise the index value would lower significantly, whereas if chloride levels rise the integrated index should show only a slight drop as it would only be a minor threat to human health. Because coliform levels have a direct implication on the health of the consumer they cannot be allowed in excess of the standards set by guidelines and standards. It has been suggested that a public drinking water supply should have a water quality index larger than 90 (with 100 being the maximum possible). The water quality would be equal to 100 if all the variables have values less than or equal to permissible levels.

4.2. Statistical Parameters for Indicator Presence

A point that needs to be considered in formulating water quality guidelines is whether arithmetic or geometric means should be used for assessing the water quality. The arithmetic mean is not considered to be a good estimator of the true arithmetic mean of coliforms in water distribution systems since large differences occur between lowest and highest values and the greater frequency of lower values is such that the distribution is skewed. These characteristics lead to an arithmetic mean that is considerably larger than the median (Standard Methods, 1987). Therefore, according to Standard Methods (1987), although regulations may require microbiological data to be reported as the arithmetic mean or median, the preferred statistic for summarizing microbiological data is the geometric mean.

4.3. Protection of Public Health in Relation to Indicator Levels

Most guidelines available are based on water quality considerations with little direct relation to human health. Epidemiological studies are of value in establishing water quality criteria based on the relationship between levels of water quality indicators and the health risk associated with the use of different water types. Epidemiological studies also maintain current awareness of the possibility of outbreaks and new aetiological agents (Craun, 1978). Only a few guidelines have been based on epidemiological considerations which mostly address recreational water quality.

Health risk assessment techniques have been proposed for the development of microbial water quality guidelines (Rose and Gerba, 1991). The goal of health risk assessment is to define levels of pollutants in water which are considered "acceptable" in terms of the human health risk they pose (Rodda, *et al.*, 1991). Risk assessment involves the identification of a health risk and the assessment of the likelihood of the risk and of its health significance. Risk assessment may be a useful tool for the formulation of water quality guidelines in the absence of epidemiological data (Rodda *et al.*, 1992, Cotruvo, 1987). While used extensively in the development of chemical water quality guidelines, the application of these techniques to microbial water quality guidelines is still relatively new. The US EPA proposed risk based guidelines for the microbial water quality of drinking water. Water treatment is required to reduce levels of *Giardia* and enteric viruses by 3 and 4 logs, respectively to achieve an annual risk of infection not greater than 1 in 10000 (Federal Register, 1989).

4.4. Steps Required Following Unsatisfactory Samples

If microbial levels are exceeded it is recommended that officials should be promptly alerted. Whenever sample results exceed set values resampling must be undertaken

immediately and no coliforms should be present in these repeat samples (SABS, 1984). Action following repeated unsatisfactory results often includes: a sanitary survey to ascertain the source of contamination; changes to alleviate the problem, which may include increased chlorine dosage, flushing water mains or using an alternative water source; and notification of the water authority and/or the public and issuing a "boiling" notice (Mc Neill, 1985). A sanitary survey consists of an on-site inspection and evaluation by a qualified person of all the conditions, devices and practices in the water supply system that may pose a risk to the health of the water consumer (WHO, 1984). Not all potential risks are equally serious.

4.5. Additional Samples and Interpretation of Results

Many guidelines recommend that additional samples be examined if the microbial quality of the water under investigation indicates the presence of bacterial contamination above specified guideline values. The percentage of sample compliance may be influenced by additional samples following an unsatisfactory sample. Pipes and Minnigh (1987) illustrate that if from five samples tested originally, one was exceeding the levels of coliforms, the frequency of noncompliance is 20%. This frequency can be reduced to 10% if five additional samples are taken and are tested negative for coliforms. If the additional samples test positive, not only has the percentage of noncompliance been maintained and possibly increased, it also clearly indicates that a problem exists.

5. SAMPLING ISSUES

5.1. Appropriate Time Period for Water Quality Evaluation

The generally accepted period of time for water quality evaluation is one month (Pipes *et al.* 1987). This is not directly related to changes in microbial water quality but rather for addressing the convenience of the administrator. Evidence indicates that although weekly or even daily changes in water quality may occur most of the time no changes occur over periods longer than one month (Pipes *et al.*, 1987).

5.2. Sample Number and Frequency

Initially, microbial water quality guidelines did not specify any requirements for a minimum number of samples to be analysed. This was due to the reasoning that the guidelines are expressed as absolute levels of safety. Presently this approach has been altered in the majority of guidelines.

The basic aim of a sampling programme is to ensure that the quality of the water sampled represents the quality of the water supply. The samples must be collected so that the analytical results represent the water quality as well as its spatial and transient variability during the time period of interest (WHO, 1987). The number of samples should be kept to a minimum to save both sampling and analytical efforts, but it is necessary to ensure that the sampling programme will readily detect

contamination. Minimum monitoring requirements recommended depend on the type of water, treatment and size of population served. The majority of guidelines and standards recommend that the frequency of sampling is dependent on the size of the population served, with larger populations needing more frequent sampling. The frequency of sampling ranges from 1 sample per month for small communities to as many as 600 samples per month for populations of greater than 4 million (Pipes, 1982; De Zuane, 1990). This principle is based on the assumption that as the population increases so will the size and complexity of the system and thus the chances of contamination by cross-connection and back-siphonage (WHO, 1984; Borup, 1992). A fraction which is allowed as positive is also selected (usually 5% of samples in a month/year). If 60 samples are examined and 3(5%) are found to be positive, then it can be said that we are 95% confident that less than 10% of the water is contaminated (Pipes, 1982). The probability of violating the maximum contaminant level can be very different for the same water quality depending on the number of samples taken (Borup, 1992). If 5% of samples are positive (or $p = 0.05$) there is a significant probability that the maximum contaminant level (MCL) will be violated. For example, if a utility needs to take 60 routine samples per month (according to the population size it serves) with $p = 0.05$ there is a 0.353 probability that the MCL will be violated by the routine samples alone. Borup (1992) also explains that there is very little benefit from a statistical point of view for taking more than 150 samples/month because the probability of violating the MCL given a particular value of p changes very little when the number of samples is greater than 150. With presence-absence testing, required by the new Total Coliform Rule in the USA, the MCL is far less likely to be violated when water quality is impaired in a localised section of the distribution system, even if a sample is taken from the section of that section of the distribution system. This is because MCL violation depends on more than 5% of the total number of samples testing positive, and it is highly likely that only one or two samples will be taken from the affected area. This may result in very high densities going undetected. Another observation is that the probability of not violating the MCL with fewer than 50 samples/month is high. Small numbers of samples taken per month will result in even very poor water quality waters going undetected and found to comply with the MCL. At least 30 samples need to be taken and zero positive samples observed to ensure the 90% confidence interval does not include $p > 0.10$. If the maximum allowable positive samples is observed it is necessary to take at least 90 samples to achieve these results.

Hayes (1989) also calculated risks of poor water going undetected and concluded that even if 52 samples over time are unsatisfactory it is still possible for unsatisfactory conditions to occur for 1% of that time period without being noticed. There is a 5% chance of unsatisfactory conditions occurring for 6% of the time period going unnoticed. Even though a standard might require zero *E. coli*, by taking 52 samples a year the actual standard being applied is a 99th percentile (Hayes, 1989).

A new source of water supply should be monitored more frequently so that variations in quantity can be observed under a variety of weather and climatic conditions. The South African SABS specifications also follow the principle of

monitoring according to the size of the population served, but add that the frequency should increase during the rainy season.

The use of the principle of frequency of sampling based on population size has been criticised because the connection between the minimum frequency of sampling and size of population served is empirical and devoid of any mathematical basis (Maul *et al.*, 1991). Sampling theory suggests that the number of samples required is related to the desired precision of the parameter estimation and not to the size of the water system (Pipes,1986). To approach the question of frequency of sampling it is assumed that the rule was intended as a limit on the average coliform density in the water distribution system. To take this one step further, it can be assumed that the intention of the rule was to limit the total number of coliforms in the system (which is equal to the mean density multiplied by the volume of the water in the system).

Maul (1991) states that a knowledge of the configuration of the heterogeneity of the distribution of bacteria in space and time within a distribution network is a vital prerequisite for the definition of the routine sampling procedures. The quantity of water analysed constitutes only a very small fraction of the quantity of water distributed. Taking the heterogeneity of the system into account implies a certain risk of arriving at an erroneous conclusion. Two types of errors (risks) may arise. The one is if the mean bacterial density in the water is below the maximum acceptable concentration according to the regulations but the results of the analysis have led to a noncompliance decision. This is termed a Type I risk, (risk α) or "producers risk". The second is when an observation conforms to a given specification, but in fact the bacterial density exceeds the maximum acceptable concentration and is termed a Type II risk (risk ß) or "consumers risk". Before a sampling regime is started it is necessary to determine the degree of heterogeneity characterizing each of the simple networks which make up the complete system under test. It is then necessary to determine the number of samples needed. This degree of heterogeneity is expressed as a value k and can be calculated using a computer programme (VOLKA). To determine the number of samples that need to be taken in a simple network in order to fulfil the condition that the risk to the consumer is below a certain threshold ß, which is decided beforehand, is given by the expression:

$$n \geq \frac{\delta t_0^2 (1 + \delta/k)}{(\mu - \delta)^2}$$ where $\delta = kp$ and is = mean bacterial density

t_0 = threshold value of the standard normal distribution corresponding to the probability level ß. This value can be obtained by reading a table of relevant values for the standard normal distribution.

The value k (expressing heterogeneity) and the value n are inversely related.

It becomes more difficult to detect infringements of the standard as the difference between the true bacterial concentration and the maximum acceptable concentration is reduced. There may be greater heterogeneity among larger distribution systems and this greater heterogeneity may be a rationale for requiring more samples for larger systems.

Examination of larger samples (200ml -1000ml) would be statistically more meaningful and would reduce the risk of failure to detect low levels of coliforms. Larger test volumes would increase the baseline sensitivity and could approach concentrations essential for control of water-borne viruses (WHO, 1984).

5.3. Sampling Site Selection

The exact site for sampling needs to be chosen with care in order to allow for samples which represent a large area covered by the distribution system. Microbial water quality guidelines can not provide exact recommendations on the selection of sampling location because of the complexity of the issues involved, therefore decisions addressing where to collect water samples for water microbiological quality analysis are often left to the judgement of local authorities. Choosing sampling sites may be based on two extreme approaches, *ie.* selection on a wholly random basis or a systematically selection based on knowledge on factors affecting microbial water quality. A knowledge of the configuration of the heterogeneity of the distribution of bacteria within a distribution network is a vital prerequisite for selecting the sampling locations. Random sampling is usually desirable when the spatial variations in quality are completely random, but it may not be ideal if there are systematic differences in quality between different parts of the distribution system. It is common to use fixed sampling locations for month to month for continuity and to allow comparisons of quality to be made.

According to Pipes and Christian (1982) the best approach for selection of sampling locations for microbiological monitoring of water in the distribution system is stratified random sampling. The water distribution system should be divided into areas in relation to locations of possible cross contamination. Thereafter, individual sampling locations in each area are selected by a random process. This approach is rarely practised due to practical problems such as inaccessibility of selected locations.

5.4. Sampling Time (*ie.* time of day; day of week)

No standard or guideline specifies the time for sample collection, *ie.* specific day of the week, or time of day. The sampling time is usually decided by the individual water authority and it is only in certain cases that monitoring is conducted according to a specified timetable. The quality of water in the distribution system may vary according to time of day and day of the week in relation to the water usage and temperature variations between day and night. Variations of temperature may be important for areas where plumbing systems are not protected.

5.5. Number and Location of Additional Samples

The majority of guidelines specify that additional samples are required following an

exceedance of the guideline. This may be interpreted as an intention to investigate possible cross-connection at the specific sampling location or possible contamination throughout the water distribution system. In some guidelines only one additional sample is specified while in others up to four additional samples are required to provide for a more thorough evaluation of the whole distribution system. These additional samples often include nearby sampling locations, not solely the point under investigation.

6. INTERNATIONAL MICROBIOLOGICAL STANDARDS AND GUIDELINES FOR DRINKING WATER

Each regulatory body has its own criteria which vary between countries (Table 1), but in a general sense all regulations are alike in stating maximum permissible concentration levels for each microbiological parameter, together with a minimum sampling frequency.

Standards generally take one of two forms:
- a maximum value for the arithmetic mean of counts observed for the group of samples analysed during a given period
- a maximum proportion for the number of samples which are allowed to exceed a stated threshold during the same period (Maul *et al.*, 1991).

In the USA the "Safe Drinking Water Act" enacted in 1974 authorized the Federal Government to establish national drinking water regulations and these regulations are enforceable. These regulations set maximum permissible levels and established monitoring requirements. In 1989 the Total Coliform Rule came into effect, and is based on the presence or absence of coliforms rather than maximum levels permissible. The maximum contaminant level is based on a certain percentage of positive samples per month rather than an estimated density of total coliforms. All samples testing positive for coliforms must be followed by repeat sampling and tested further to determine whether faecal coliforms are resent. (AWWA, 1990). No more than 5% of monthly samples may be positive if >40 samples are analysed each month, or no more than 1 sample may be positive if <40 samples are analysed each month. All positive samples including repeat samples must be included in compliance testing.

Special attention has been focused on *Giardia*, viral agents, *Legionella* and opportunistic pathogens. Recommendations for additional treatment processes to control these pathogens have been made by stating that the treatment process must provide for a 3 and 4 log reduction of *Giardia* and viruses respectively (AWWA, 1990).

The European Community (EC) Drinking Water Directive (1980) specifies 66 requirements for which standards are set, which include the microbiological parameters total and faecal coliforms, faecal streptococci, sulphide-reducing clostridia and total bacterial counts. Water intended for human consumption also

should not contain pathogenic organisms such as staphylococci, salmonella, faecal phage and enteroviruses, parasites, algae and "animalcules" (Chiaudani and Premazzi, 1988). Three levels of standards are used, *ie.* the maximum admissible concentration (MAC), the minimum required concentration (MRC) and the guide level (GL). The member state must set values for all the parameters and the values may not be higher than the MAC or lower than the MRC. If only guide levels are quoted in the directive, values are set by member states at their discretion. The European countries which follow the EC Directive for faecal and total coliforms, faecal streptococci and the sulphite reducing clostridia (1980) include Denmark, Germany, Greece, Italy, Luxembourg, Netherlands, Belgium, Portugal, the UK and Ireland (Ref. 1989). Belgium has set more stringent guidelines in that they require a larger sample volume to be examined (250ml compared to the recommended 100ml) and they also require the absence of *Pseudomonas aeruginosa*. For the total bacterial counts the countries vary in the counts permitted per 1ml from 2-20/ml at 37°C and 20-100/ml at 22°C. Total bacterial counts are not considered in French legislation. Some countries require that only the maximum allowable concentration (MAC) is not exceeded while others specify both the guide level and the MAC. In 1987 the EC officials stated that the MAC was a concentration that should not be exceeded in individual samples, but EUREAU (European Union of Water Undertakers) would prefer to see a percentile approach adopted for parameters not related to health (Carney, 1991). This is in line with the WHO 1984 guideline values, where short term exceedances of guide levels does not necessarily mean that the water is unsuitable for consumption.

The most lax of all international standards are those of the WHO which have been devised to accommodate 3rd world countries. The WHO (1984) states that the adoption of too stringent drinking water standards could limit the availability of water supplies that meet those standards. This is a significant consideration in regions of water shortage, such as South Africa. The primary aim of the WHO drinking water guidelines is the protection of public health. The guideline values represent target quality objectives and have been derived to safeguard health on the basis of lifelong consumption. Short term deviations above the guideline values do not necessarily mean that the water is unsuitable for consumption. Emphasis is placed on general source protection to minimize health problems from biological agents in drinking water supplies. The microbial quality of drinking water is considered to be of the greatest importance and must never be compromised to provide acceptable water. The primary bacterial indicator recommended for the examination for indicators of faecal pollution is the coliform group. The detection of faecal coliforms, in particular *E. coli* provides definite evidence of faecal pollution. The WHO guidelines were updated and revised recently (1993) and it is now recommended that no *E. coli* or thermotolerant coliforms be detectable in any 100mℓ sample for all water intended for drinking or treated water entering the distribution system. Treated water within the distribution system should not have any thermotolerant coliforms or *E. coli* and total coliforms must not be present in 95% of samples taken throughout any 12 month period. Previously, the WHO (1984) specified that the presence of not more than 3 coliform organisms/100ml may be tolerated in occasional samples provided that faecal coliform samples are absent. For large supplies it was recommended that throughout any 1 year period

no coliform organisms be detected in 98% of all routine samples. In addition coliform organisms should not be detected in any 2 consecutive routine samples. Drinking water should also be free from any viruses and parasites infectious for man.

Australia has no national legislation governing the quality of drinking water as this is controlled by legislation of each state. The Australian guidelines for drinking and other domestic purposes states that water should be safe, palatable and aesthetically pleasing. Two types of limits have been proposed (Hart, 1974), namely, objective levels and derived working levels, or maximum permissible levels. The Treated drinking water supplies should have 0 total and faecal coliforms and *E. coli*/100ml. For untreated supplies a 3 tier system exists stating whether the water is "satisfactory, suspicious or unsatisfactory". Guidelines have been drafted (Hart *et al.*, 1992) which apply to the quality of the raw water. Water that is to be disinfected may have higher microbial guideline values. Water that is to be subjected to coarse screening only may have 10 total coliforms /100ml but no two consecutive samples should be coliform positive and 95% of samples annually should be free of coliforms in 100ml. No samples should contain faecal coliforms. Water that is subjected to coarse screening and disinfection should contain less than 100 coliforms /100ml and less than 10 faecal coliforms /100ml in 95% of samples (Hart *et al.*, 1992).

The Canadian Guidelines for drinking water are also not legislated and may be modified according to local conditions. Generally the 1978 guidelines are thought to be adequate in the case of microbiological parameters for protecting human health (Toft *et al.*, 1987; Canadian Water Quality Guidelines, 1992). The guidelines state that no sample should contain more than 10 total coliforms/100ml and that not more than 10% of samples taken in a 30 day period should be coliform positive. No more than 2 consecutive samples should be coliform positive and no faecal coliforms should be detected in 100ml sample. It is also suggested that raw water coliform measurements be used to assist in determining treatment requirements. For example, if faecal coliform densities are greater than 100/100ml or if total coliform counts are greater than 1000/100ml, the water should receive complete treatment. All supplies derived from surface water should receive disinfection as a minimal treatment (Canadian Water Quality Guidelines, 1992).

In the United Kingdom, the EC directive mandatory limits for drinking water quality have been implemented since 1985 (Hayes, 1989). Because of the epidemiological evidence from Australia suggesting a possible link between toxin producing strains of *Aeromonas sp.* and outbreaks of gastroenteritis, the Governmental Standing Committee of Analysts in the UK is reviewing the low priority given to bacterial counts. Water undertakings in the Netherlands have also adopted tentative guidelines for *Aeromonas sp.* of less than 20/100ml as a 90 percentile within one month for final drinking water (Hayes, 1989).

The Israeli National Health Act of 1974 makes provision for the protection and analysis of drinking water. No faecal coliforms may be present in 100ml volume. If greater than 10 total coliforms or any faecal coliforms are detected in 100ml of

water then the water must be reanalysed within 3-10 days, depending on the numbers detected. If 3-10 total coliforms/100ml are detected, the water must be analysed within 10 days, and if >10 coliforms/100ml are detected the water must be reanalysed within 3 days. If repeat tests are found to contain coliforms a sanitary survey must be conducted and the health authorities notified immediately.

In Japan, the Waterworks Law requires that water supplied by waterworks shall meet the drinking water quality standards prescribed, which state that the water must not be affected by any pathogenic organism, nor contain any organism which gives grounds for suspicion of being contaminated by a pathogenic organism. Zero total coliforms (no volume is specified) and a maximum bacterial count of 100/ml are allowed. Free residual chlorine concentrations must be ≥ 0.1ppm to guarantee tap water free from pathogenic microorganisms (Magara and Morishita, 1988).

7. APPLICABILITY OF MICROBIOLOGICAL DRINKING WATER GUIDELINES TO THE SOUTH AFRICAN SCENARIO

In order to evaluate the applicability of various international guidelines and evaluate available local guidelines there is a need to firstly discuss the South African scenario. South Africa represents a mix of developed and developing communities. This mixture includes highly developed and structured urban areas in which industry plays a major role. Such structured urban regions often are accompanied with peri-urban areas where only limited water supply and sanitation infrastructures are available. In addition, a large sector of the population reside in rural areas where no formal water supply is provided. In summary, although part of the South African population has access to drinking water of high quality a large number of South Africans have either limited or no access to safe water. According to Aucamp (1993) it is estimated that in South Africa almost 11 million people (of which 7 million are living in rural environments) do not have a safe water supply. The increasing population growth and urbanisation is placing pressure on the need for safe water supplies, but both financial and human resource constraints can affect the rate in which such infrastructures can be introduced. Three main issues govern the South African water scenario, *ie.* water quality, water availability and water pollution. Often the three overlap and relate to each other. In situations where no proper water supplies are provided the situation coincides with no sanitation resulting in increased pollution of water resources.

In the light of this scenario the importance of microbial water quality guidelines can not be over-emphasised. Such guidelines should not only address high quality purified potable water, but also be appropriate for areas in which only localised purification schemes and limited infrastructures for water supplies are available. However, only water supplied to the consumer should be evaluated as drinking water. To develop guidelines for communities where no water supplies are available and surface waters are used will require an holistic approach to surface water management in which pollution sources are well managed. Under the conditions where no sanitation provisions are evident, such as in many peri-urban developments, any attempt to manage non-point source faecal pollution will be

complex.

At present South African water quality guidelines are not legally enforceable. The South African Bureau of Standards (SABS) has provided specifications or guidelines for water for domestic supplies (SABS-241, 1984) providing 2 tiered limits for water fit for human consumption. The limits are based on those issued by the WHO, the EC and US-EPA. The limits are specified as either "recommended" or "maximum allowable". The recommended limit should be applied to all water supplies and the "maximum allowable" limit should never be resorted to unless no other water supply is practically available. The microbiological parameters specified include total and faecal coliforms as well as standard plate counts (See Table 1 for a comparison of international drinking water quality parameters). Another set of drinking water quality criteria was proposed in 1990, based on a 3 tiered system providing for a wide variety of local conditions (Aucamp and Vivier, 1990). The first tier is the ideal level, or maximum level for no risk. It closely follows the recommended levels set by the American EPA, EEC, WHO and SABS. Drinking water conforming to these maximum levels is considered to be safe for a lifetime's consumption and is regarded as the "no risk range". The 2nd tier is the "maximum level for insignificant risk" and is similar to levels of maximum permissible levels at which human health risk is considered to be negligible even during a lifetime's consumption. It is the lowest quality of water acceptable under normal circumstances. The 3rd tier represents the "maximum level for low risk". It was designed to provide water for the short term use of water of undesirable quality without creating an unacceptably high health risk. These values are usually double those of the 2nd tier, and should not occur for more than 2 consecutive days or for a total of 12 days a year without reporting the case to the authorities.

The rationale used in setting this 3 tiered system is that with a single criterion the limit is often regarded as a law which may under no circumstances be exceeded. Risks associated with deteriorating water quality increase gradually, rather than changing from acceptable to completely unacceptable at a particular limit. This system offers a more realistic approach and it administers the concept of "health risk ranges". It is believed that with this system unpredictable situations which require a less rigid water quality control system can be controlled to meet arising emergencies efficiently (Aucamp and Vivier, 1990).

The latest guidelines published for South Africa are those of the DWA&F (1993). This document is intended as a guide document to be used for the development of limits for managing water resources for particular areas and water uses. They are also based on a range of values, as are those of Aucamp and Vivier (1990), where each range is associated with a description of fitness for use, and where the total range extends from the most ideal to the point of unacceptability. Guideline values are given for microbiological parameters including faecal coliforms/*E. coli*, coliphages, protozoan parasites and enteric viruses. Guideline values have not been included for standard plate count or total coliforms in the first edition of this guideline document. It is the first South African guideline document to recommend analyses for protozoan parasites on a low frequency basis.

ould not contain pathogenic organisms such as staphylococci, salmonella, faecal age and enteroviruses, parasites, algae and "animalcules" (Chiaudani and mazzi, 1988). Three levels of standards are used, *ie.* the maximum admissible ncentration (MAC), the minimum required concentration (MRC) and the guide el (GL). The member state must set values for all the parameters and the values ay not be higher than the MAC or lower than the MRC. If only guide levels are oted in the directive, values are set by member states at their discretion. The ropean countries which follow the EC Directive for faecal and total coliforms, ecal streptococci and the sulphite reducing clostridia (1980) include Denmark, rmany, Greece, Italy, Luxembourg, Netherlands, Belgium, Portugal, the UK and land (Ref. 1989). Belgium has set more stringent guidelines in that they require arger sample volume to be examined (250ml compared to the recommended 0ml) and they also require the absence of *Pseudomonas aeruginosa*. For the total cterial counts the countries vary in the counts permitted per 1ml from 2-20/ml 37°C and 20-100/ml at 22°C. Total bacterial counts are not considered in French gislation. Some countries require that only the maximum allowable concentration IAC) is not exceeded while others specify both the guide level and the MAC. In 87 the EC officials stated that the MAC was a concentration that should not be ceeded in individual samples, but EUREAU (European Union of Water ndertakers) would prefer to see a percentile approach adopted for parameters not lated to health (Carney, 1991). This is in line with the WHO 1984 guideline lues, where short term exceedances of guide levels does not necessarily mean at the water is unsuitable for consumption.

ne most lax of all international standards are those of the WHO which have been vised to accommodate 3rd world countries. The WHO (1984) states that the loption of too stringent drinking water standards could limit the availability of ater supplies that meet those standards. This is a significant consideration in gions of water shortage, such as South Africa. The primary aim of the WHO inking water guidelines is the protection of public health. The guideline values present target quality objectives and have been derived to safeguard health on e basis of lifelong consumption. Short term deviations above the guideline values not necessarily mean that the water is unsuitable for consumption. Emphasis is aced on general source protection to minimize health problems from biological gents in drinking water supplies. The microbial quality of drinking water is nsidered to be of the greatest importance and must never be compromised to ovide acceptable water. The primary bacterial indicator recommended for the amination for indicators of faecal pollution is the coliform group. The detection faecal coliforms, in particular *E. coli* provides definite evidence of faecal llution. The WHO guidelines were updated and revised recently (1993) and it is w recommended that no *E. coli* or thermotolerant coliforms be detectable in any 00mℓ sample for all water intended for drinking or treated water entering the stribution system. Treated water within the distribution system should not have ny thermotolerant coliforms or *E. coli* and total coliforms must not be present in 5% of samples taken throughout any 12 month period. Previously, the WHO 984) specified that the presence of not more than 3 coliform organisms/100ml ay be tolerated in occasional samples provided that faecal coliform samples are sent. For large supplies it was recommended that throughout any 1 year period

8. FUTURE TRENDS

Health risk assessment techniques have been proposed for the development of microbial water quality guidelines (Rose and Gerba, 1991). The goal of health risk assessment is to define levels of pollutants in water which are considered "acceptable" in terms of the human health risk they pose (Rodda, *et al.*, 1991). Risk assessment involves the identification of a health risk and the assessment of the likelihood of the risk and of its health significance. Risk assessment may be a useful tool for the formulation of water quality guidelines in the absence of epidemiological data (Rodda *et al.*, 1992, Cotruvo, 1987). Risks may be estimated prospectively where no historical data exists and if the risk is small. The US - EPA (1987) has proposed a microbial drinking water guideline based on risk. It requires the reduction of *Giardia* and viruses by 3 and 4 logs respectively in a water treatment process to ensure less than a 10^{-4} (1 in 10 000) annual risk of infection (Federal Register, 1989).

The standard for an individual country must be based on a study of the health risk and factors relating to water scarcity, climatic conditions and technological and economical feasibility. The feasibility of applying microbial risk assessment techniques are currently being examined in South Africa by the Health Programme of the Division of Water Technology, CSIR, by assessing risks associated with enteric viruses in raw and treated drinking water (Rodda *et al.*, 1992). Application of risk assessment techniques clearly showed the volume of water monitored to be the most important factor limiting detection of low risk levels. The sampling and concentration of large volumes of at least 100ℓ in size was urgently required.

9. CONCLUSIONS AND RECOMMENDATIONS

It is of limited value to recommend water quality guidelines without also coupling these guidelines to the methods and frequency of sampling and analysis required to adequately monitor and assess the water quality. It is likely that water monitoring will change with the introduction of statistical approaches to quality control and risk analysis.

South African guidelines should be revised, particularly with regards to the inclusion of limits for pathogens and the latest methodologies for the detection of both indicator organisms and pathogens such as enteric viruses and protozoan parasites. The DWA&F guidelines have included coliphages as indicators of enteric viruses, but mention the limitations on their usefulness as viral indicators as well as advising that the guideline should be considered as extremely tentative.

Although the need for additional indicator organisms is not recommended in the light of increased non-point source pollution it would be of benefit for the health of the South African population if water supplies are occasionally evaluated for enteric viruses, or indicators for viral pollution such as bacteriophages and the presence of protozoan parasite cysts and oocysts. These parameters are especially

important where water purification processes applied are limited. It is recommended that the sample volumes (as proposed by Aucamp and Vivier, 1990 and DWA&F, 1993 (Table 1)) of 10ℓ be increased to 100ℓ for enteric viruses and protozoan parasites. This recommendation is based on a risk assessment conducted on microbiological monitoring data of South African waters.

It is believed that the present indicator organisms recommended in the SABS specifications are adequate for the routine monitoring of drinking water, but if problems are suspected then additional tests should be specified, for example, testing for the presence of *Vibrio cholerae* and *Salmonella spp.*, in areas where non-point source pollution occurs and examination of water for *Aeromonas spp.* and *Pseudomonas,* depending on the type of water and distribution system. It is also believed that water should be examined for the presence of enteric viruses and protozoan parasites, such as *Giardia* and *Cryptosporidium* on a routine but less frequent basis (*eg.* monthly).

Meaningful statistical descriptions of data processing needs to be addressed in South African guidelines. Statistical aspects of water quality monitoring, for instance the monitoring frequency and data analysis, ie. central tendency and variability. For example, whether to use the arithmetic or geometric mean as measures of central tendency and whether to use the standard deviation, 90th percentiles or 90% confidence intervals as the measure of variability. The monitoring frequency should be re-examined in light of data available demonstrating that there is very little benefit from a statistical point of view for taking more than 150 samples/month and fewer than 50 samples/month. Small numbers of samples taken per month may result in poor water quality waters going undetected. The poor water quality is no less serious to inhabitants of a city with a population of less than 500 000 than it is to inhabitants of a city with a population of greater than 500 000. (The SABS specifications recommend a monitoring frequency of 50 samples per month only for distribution systems serving greater than 500 000 people). It has been calculated that at least 30 samples need to be taken and zero positive samples observed to ensure the 90% confidence interval does not include $p > 0,10$. The principle of frequency of sampling based on population size has been criticised because it lacks any mathematical basis.

It will be necessary to establish a task group for the assessment and re-evaluation of drinking water quality guidelines for South Africa. The task group should include members of governmental departments concerned with water quality and health, organisations actively involved in microbiological aspects of drinking water quality, as well as major water boards and authorities. In addition to re-assessing the guidelines the task group should also examine the feasibility of establishing drinking water *standards* for microbiological quality of drinking water in South Africa.

10. REFERENCES

Anon, 1990. Drinking Water Standards in South Africa. IMIESA **15** (11), pp 9-17.

APHA, 1980. Standard Methods for the Examination of Water and Wastewater., 15th ed., APHA-AWWA-WPCF, Washington DC., USA.

Aucamp, P.J. and Vivier, F.S., 1990. Water Quality Criteria in South Africa. Technology SA, p21-30.

Aucamp, P.J. 1993. Disinfection of and disinfection by-products in drinking water in South Africa - A national report. Presented at IWSA Conference, Budapest.

AWWA, 1990. Total Coliforms. - A working explanation of the total coliform rule. Safe drinking water act series.

Berg, G., 1978. The indicator system, *In* Berg, G. (Ed.), Indicators of Viruses in Water and Food, Ann Arbor Sci. Mich.

Bhargave, D.S., 1985. Expression for Drinking Water Supply Standards. J. Environmental Eng. **111**,(3), 304-316.

Borrego, J.J., Morinigo, M.A., de Vincente, A., Corax, R. and Romero, P., 1987. Coliphages as an indicator of faecal pollution in water. Its relationship with indicator and pathogenic microorganisms. Wat. Res. **21**, (12), 1473-1480.

Borup, M.B., 1992. Presence-absence Coliform Monitoring Has Statistical Limitations. J.A.W.W.A., 1992, pp 66-71.

Bourne, P., 1982. Rural Water Supply and Health. ed. Falkenmark, M. Ch 2, pp. 35. Uppsala: Scandinavian Institute of African Studies.

Boyd,S., Jones, A., Knaust, A. and Mc Grath, C., 1986. Drinking Water: A community Action Guide. CONCERN INC.

Canadian Guidelines (1987). Guidelines for Canadian Drinking Water Quality. Minister of National Health and Welfare No. 1148-10/1987.

Canadian Water Quality Guidelines, (1992). CCME Canadian Water Quality Guidelines, prepared by the Task Force on Water Quality Guidelines of the Canadian Council of Resource and Environment Ministers, April, 1992.

Carney,M., 1991. European Drinking Water Standards. JAWWA, June, pp48-55.

Chiaudanis, G. and Premazzi, G., 1988. Water Quality Criteria in Environmental Management. Environment and Quality of Life. EUR 11638 EN.

Clark, D.L., Milner, B.B., Stewart, M.H., Wolfe, R.L. and Olson, B.H., 1991. Comparative Preparations with the Standard Methods MF Faecal Coliform Test for the Detection of *E. coli* in Water Samples. Appl. & Environ. Microbiol., 57,(5), 1528-1534.

Cotruvo, J.A. 1987. Risk assessment and control decision fro protecting drinking water quality. *In:* Advances in Chemistry, No. 214, J.H. Suffet and Murugan Maliyandi, (Eds.). American Chemical Society, Washington DC, USA, 693-733.

Craun, G.F., 1978. Impact of the coliform standard on the transmission of disease. *In* Hendricks, C.W. (Ed), 1978.

De Wet, C.M.E., Grundlingh, M., Louw, B. and Bester, D., 1991. Evaluation of faecal enterococci isolation media to indicate faecal pollution in chlorinated water. Wat. Sci. Tech. **24** (2), pp.77-80.

De Zuane, J., 1990. Drinking Water Quality. Standards and Controls. Van Nostrand Reinhold, New York.

DHSS, 1969. Reports on Public Health and Medical Subjects No. 71. The Bacteriological Examination of Water Supplies. Dept. Health and Social Services, Welsh Office, Ministry of Housing and Local Government, HMSO, London.

Department of Water Affairs and Forestry (DWA&F), 1993. South African Water Quality Guidelines. Volume 1: Domestic Use.

EEC Council Directive, 1980. Relating the quality of water intended for human consumption. L229, 80/778/EEC.

Federal Register, 1989. National Drinking Water Regulations: Filtration, Disinfection, Turbidity, *Giardia lamblia*, Viruses, *Legionella* and Heterotrophic Bacteria. Final Rule, 40 CFR. *US Federal Register*, **54**, June 29 1989, 27486-27541.

Geldreich, E.E., Allen, M.J. and Taylor, R.H., 1978. Interferences to coliform detection, p13-20. *In* Hendricks, C.W. (ed.) 1978.

Grabow, W.O.K., 1991. Human viruses in water. Water Sewage and Effluent, **11(4)**, 16-21.

Hart, B.T., 1974. A compilation of Australian water quality criteria, AWRC Technical Paper No. 7 AGPS, Canberra.

Hart, B.T., Angehrn-bettinazzi, C., Campbell, I.C. and Jones, M.J., 1992. Australian Water Quality Guidelines, *In preparation*

Hayes, C.R., 1989. Microbiological Quality Control in the Provision of Safe Drinking Water. Wat. Sci. Tech., **21**, 559-566.

IAWPRC Study Group on Health Related Water Microbiology. 1991. Reveiw paper: Bacteriophages as model viruses in water quality control. Water Research **25(5)**, 529-545.

Inhaber, H., 1976. Environmental Indices. Wiley-Interscience Publication. New York.

ISO, (International Standardization Organisation) 1980. Water quality - sampling Part 1. ISO, Switzerland.

ISO, 1982. Water quality sampling - Part 2. ISO, Switzerland.

Kfir, R., Hilner, C. and Du Preez, M., 1993. The occurrence of protozoan parasites in South African water. Proc. Third Biennial WISA conference, Durban, 24-26 May, Vol 2, pp 47-59.

Kibbey, H.J., Hagedorn, C., and McCoy, E.L., 1978. Use of faecal streptococci as indicators of pollution in soil. Appl. Environ. Microbiol. **35**, pp711-717.

Kott, Y. 1984. Coliphages as reliable enteric virus indicators. *In Enteric Viruses in Water* (Ed. Melnick), Karger, Basel.

Levin, M.A., 1977. Bifidobacteria as water quality indicators, *In* Hoadley, A.W. & Dutka, B.J. (ed.) Bacterial indicators/health hazards associated with water. Special Tech. Publ. 635, Philadelphia.

Lewis, W., 1985. The Significance of Water Management in Relation to Public and Environmental Health. J. Appl. Bacteriol. Symposium Supplement. 1S-13S.

Magara, Y. and Morishita, T. 1988. Water supplies for cities and industries. Water Resources Development, Vol.4 (1), pp 30-34.

Maul, A., Vagost, D., Block, J.C., 1991. Microbiological Analysis in Water Distribution Networks. Ed.Winkler, M. Ellis Horwood Ltd., West Sussex, England.

McFeters, G.A., Pyle, B.H., Gillis, S.J., Acomb, C.J. and Ferrazza, D., 1993. Chlorine injury and the comparative performance of Colisure, Colilert and Coliquik for the enumeration of coliform bacteria and *E. coli* in drinking water. Wat. Sci. Tech. **27** (3-4), 261-265.

McNeill, A.R., 1985. Microbiological Water Quality Criteria:A Review for Australia, Australian Government Publishing Service, Canberra.

Morinigo, M.A., Wheeler, D., Berry, C., Jones, C., Munoz, M.A., Cornax, R. and Borrego, J.J., 1992. Evaluation of different bacteriophage groups as faecal indicators in contaminated natural waters in southern England. Wat. Res., **26** (3), 267-271.

Olson, B.H., Clark, D.L., Milner, B.B., Steward, M.H., and Wolfe, R.L., 1991. Total Coliform Detection in Drinking Water: Comparison of Membrane Filtration with Colilert and Coliquik. Appl. Environ. Microbiol, **57**, (3), pp1535-1539.

Pipes, W.O. and Christian, R.R. 1982. Sampling frequency-microbiological drinking water regulations, EPA-570/9-82-001. Office of Drinking Water, US-EPA, Washington DC.

Pipes, W.O. 1986. Statistical Inferences from Coliform Monitoring of Potable Water. pp 183-193, In *Statistical Aspects of Water Quality Monitoring Workshop*, Developments of Water Science, Vol. 27. Ed. Elshaarawi A.H. *et al.*

Pipes, W.O. and Minnigh, H.A. 1987. Significance and interpretation of repeat sampling results. Technical Conference Proceedings, *Advances in water analysis and treatment*, paper ST-11, AWWA, Denver Colorado.

Pipes, W.O., Mueller, K., Troy, M.A. and Minnigh, H.A. 1987. Frequency-of-occurrence monitoring for coliform bacteria in small water systems. JAWWA **79**, 59-63.

Pontius, F.W., 1992. A Current Look at the Federal Drinking Water Regulations. J.A.W.W.A, 1992, pp 36-50.

Reitler, R. and Seligmann, R., 1957. *Pseudomonas aeruginosa* in drinking water. J. Appl. Bacteriol., **20**, 145-150.

Rodda, N., Genthe, B., Slabbert, J.L. and Kfir, R. 1991. Health implications of water pollution. The role of research. Paper presented at Southern African International Conference on Environmental Management, October, Somerset West.

Rodda, N., Amory, A. and Kfir, R. 1992. The application of risk assessment techniques to microbial monitoring data: A South African perspective. Paper presented at the IAWPRC conference, Washington DC, May 1992.

Rose, J.B. and Gerba, C.P., 1991. Use of risk assessment for development of microbial standards. Wat. Sci. Tech., **24**(2), pp. 29-34.

SABS, 1984. Standard Methods SABS-241.

SABS, 1990. Standard Methods SABS-221

Slade, J.S., 1985. Viruses and drinking water, J.I.W.E.S., **39**, (1), 71-80.

Standard Methods, 1989, Standard Methods for the Examination of Water and Wastewater. 17th Edition. Ed. Clesceri, L.S.; Greenberg, A.E. and Trussel, R.R., Washington DC

Tobin, R., 1988. Criteria for the microbiological quality of well water in Canada.

First Biennial Water Quality Symposium, Banff Alberta, August 29 - September 2.

Toft, P., Malaiyandi, M., Hickman, J.R., 1987. Guidelines for Canadian Drinking Water Quality, American Chemical Society.

US-EPA, 1987. The risk assessment guidelines for 1986. EPA/600/8-87/045/ US EPA Office of Health and Environmental Assessment, Washington DC, USA.

Watkins, J., and Cameron, S.A., 1991. Recently Recognized Concerns in Drinking Water Microbiology. J.I.W.E.M. (5). p624.

Wheater, D.W.F., Mara, D.D. and Oragui,, J., 1979. Indicator systems to distinguish sewage from stormwater runoff and human from animal faecal material, *In* James, A. and Evison, L. (ed) Biological indicators of water quality, John Wiley & Sons, Chichester.

WHO, 1982. Guidelines for Drinking Water Quality. Vol. 1 Recommendations

WHO, 1984. Guidelines for Drinking Water Quality. Vol. 2 Health Criteria and Other Supporting Information. Geneva.

WHO, 1987. Drinking Water Quality and Health Related Risks. Copenhagen.

WHO, 1993. Guidelines for Drinking Water Quality. 2nd Edition. Vol. 1 Recommendations

Table 1: Comparison of Microbiological Parameters used for Drinking Water Guidelines and Standards Throughout the World

Country / Microbiological parameter	South Africa 1984 SABS Specifications	South Africa - 1990 DNH&PD Guidelines proposed by Aucamp & Vivier **	South Africa - 1993 DWA&F## Water quality guidelines domestic use (suggested guidelines - 1st edition)	WHO International Guideline - 1984	WHO International Guideline - 1993
Total coliforms	0/100mℓ recommended and 5/100mℓ maximum	0/100mℓ for max. level for no risk; 5/100mℓ for max. level for insignificant risk	not included in 1st edition	0/100mℓ in 95% of samples in a year in the distribution system	0/100mℓ in 95% of samples in a year in the distribution system
	no more than 5% samples annually may be coliform +	100/100mℓ max. for low risk		3/100mℓ maximum in piped water and 10/100mℓ in unpiped water	0/100mℓ in treated water entering the distribution system
	If coliform + immediate resampling necessary			no 2 consecutive samples with coliforms	
Faecal coliforms/ *E. coli*	0/100mℓ	0/100mℓ; 1/100mℓ & 10/100mℓ for the 3 levels of risk	0/100mℓ;10/100mℓ & 20/100mℓ for the 3 levels of risk	0/100mℓ	0/100mℓ
Bacterial counts	100/mℓ	<100/mℓ; 1000/mℓ & 10000/mℓ for the 3 levels of risk	not included in the 1st edition		
Other indicators and pathogens		*Clostridium perfringens* : 0/100mℓ; 1/100mℓ & 100/100mℓ for the 3 levels of risk; Coliphages: 0/100mℓ; 10/100mℓ & 100/100mℓ; Enteric viruses: 0/10ℓ; 1/10ℓ & 10/10ℓ for the 3 levels of risk	Coliphages: 0/100mℓ; 10/100mℓ & 100/100mℓ; Enteric viruses: 0/10ℓ; 1/10ℓ & 10/10ℓ for the 3 levels of risk; Protozoan parasites: <1 *Giardia* or *Cryptosporidium* cyst/oocyst/10ℓ	<1pfu/ℓ enteric viruses	
Sampling frequency	According to population size served			Depends on water source, treatment and size of population served	

** Guidelines based on a 3 tier system: The 1st tier is the ideal level; the 2nd tier is the lowest quality of water acceptable under normal circumstances; the 3rd tier values should not occur for more than 2 consecutive days; ## Based on a tiered system ranging from ideal to unacceptable.

Table 1 cont.

Country / Microbiological parameter	EC Directive 1980 Mandatory Limits	Unite States - 1989 Safe Drinking Water Act (Total Coliform Rule)	Canadian Guidelines 1978 &1992 (non-enforceable)	Australia 1974 Guideline	United Kingdom 1969 (non-enforceable)
Total coliforms	0/100m*l* in 95% of samples annually	presence-absence test	no sample>10/100m*l*	0/100m*l* in 95% of samples annually	0/100m*l* recommended and 10/100m*l* maximum
	no sample >3/100m*l*	no more than 5% samples in 1 month may be +	no more than 10% of samples in 30d may be coliform +	no sample > 10/100m*l*	no 2 consecutive samples should be coliform +
			no 2 consecutive samples with coliforms	no 2 consecutive samples with coliforms	
Faecal coliforms	0/100m*l*	0/100m*l*	0/100m*l*	0/100m*l* and 0/100m*l* *E. coli*	0/100m*l* with a maximum of 2/100m*l* *E. coli*
Bacterial counts / standard plate count	100/1m*l*@22°C,3d 10/1m*l* @37°C,1-2d		<500/m*l* if untreated - geometric mean of >10 monthly samples		
Other indicators and Pathogens	zero faecal streptococci and a maximum of 5/100m*l* *Clostridium* Zero *Salmonella*, faecal bacteriophage and enteric viruses	*Giardia*, *Legionella*, standard plate count and viruses controlled by treatment technique	0 enteric viruses per 1000*l* desirable		
Sampling frequency	According to population size served	According to population size served	Depends on size of system, quality of source water and past history of water quality	According to population size served	According to population size served

Table 1 cont.

Country/Specifications Microbiological parameter	Japanese Waterworks Law (Magara & Morishita, 1988)	Israeli Standards 1974			
Total coliforms	Zero total coliforms (no volume specified)	If 3-10 total coliforms detected resample within 10 days			
		If > 10 coliforms detected resample within 3 days			
		If repeat samples are coliform + then a sanitary survey necessary			
Faecal coliforms		0/100ml			
Bacterial counts / standard plate count	maximum 100/ml				
Other indicators and Pathogens	No pathogens or any organisms giving grounds for suspicion of contamination				
Sampling frequency					