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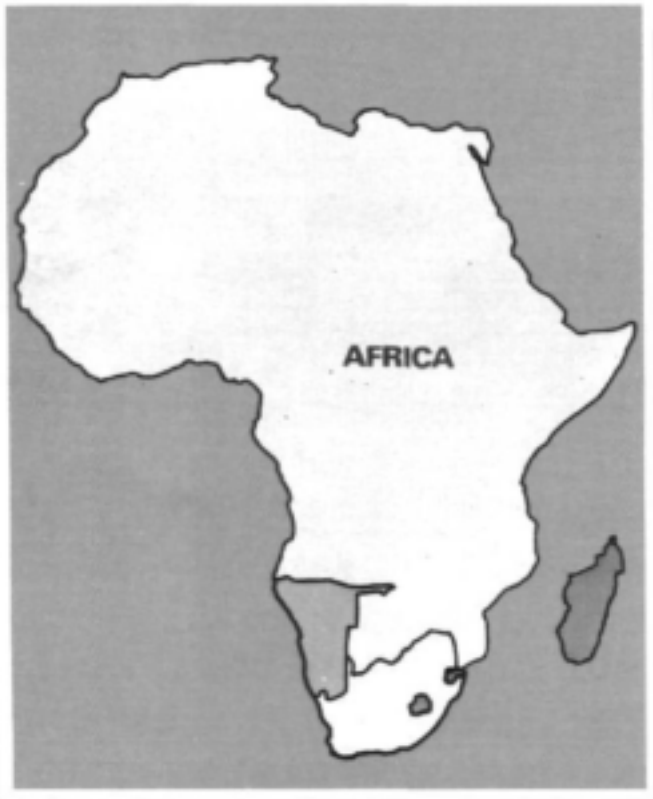

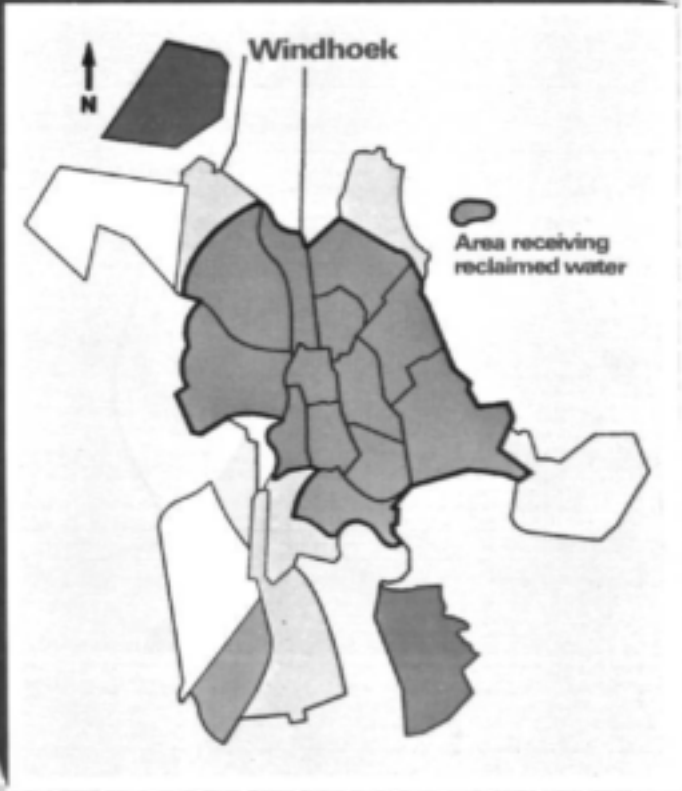

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STUDIES ON HEALTH ASPECTS OF WATER RECLAMATION DURING 1974 TO 1983 IN WINDHOEK, SOUTH WEST AFRICA/NAMIBIA

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TABLE OF CONTENTS

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FOREWORD

It is inevitable that the continued population growth and industrial expansion would result in a decreasing availability in the amount of unpolluted fresh water to be used as a source for potable water. Potable water forms part of man's diet and therefore the quality of such water is of prime importance to ensure that this water is not detrimental to the health of the consumer. South Africa suffers from a scarcity of water and therefore the use and reuse of water for more than one purpose offers an attractive means to counteract the effects of the scarcity of water on the country. However, industrial pollution and in particular the practice of the release of synthetic chemical compounds to receiving streams, carry in itself a mechanism for self-destruction in that those same compounds may cause irreparable damage to the health of the consumer. Therefore it is of paramount importance that these compounds must either be totally removed from drinking water or that the concentration thereof be reduced to such a level that the risk of exposure is reduced to as low a level as possible. To study the effects of possible exposure to these compounds it became imperative that epidemiological studies be carried out to watch over the health of the population at risk. Therefore an expert committee of the World Health Organization called for such studies to be carried out before any source of polluted water is used as a source for potable water. This call was further emphasized by the South African Department of National Health and Population Development by stipulating in their guidelines for the reclamation of water for unrestricted reuse from treated waste water, that epidemiological studies must be carried out before and after the introduction of such water.

With the advent of the introduction of reclaimed water in the city of Windhoek in South West Africa/Namibia in 1969 to overcome the effects of a serious water shortage as a result of a prolonged drought, the Water Research Commission invited the South African Institute for Medical Research to conduct epidemiological studies in Windhoek.

This document is then an overview of the studies carried out over a decade in Windhoek to assess both the short-term and long-term effects of exposure to potable water reclaimed from treated waste water. I trust that this document will serve as a basis for assessing the possible health risks involved and that it will stimulate further research into this field. My personal thanks to all the researchers who, over so many years, have spent much time and effort to ensure that the public is not exposed to undue risk and that the quality of their life could be maintained under adverse climatic conditions.

P E ODENDAAL
EXECUTIVE DIRECTOR
WATER RESEARCH COMMISSION

ACKNOWLEDGEMENTS

In the first instance we wish to extend our appreciation to Dr G J Stander, former Chairman of the Water Research Commission,whose vision and farsightedness encouraged studies which would be of benefit to human health in regard to water reclamation as well as in its broadest sense and the sustained funding by the Water Research Commission over a decade.

The authors very warmly acknowledge the assistance of several individuals who collected the data and carried out the laboratory investigations which form the backbone of this 10-year investigation and who rendered valuable advice and other assistance. As no one person can be singled out as having made a less important contribution than another, they are listed in alphabetical order as follows :

Sister MJ Bouwer, Professor JHS Gear, Dr SS Grovè, Ms SJ Hall who was responsible for the artwork, Mrs E Hay and Mrs C Thompson who typed the manuscript, Dr KP Klugman, Professor HJ Koornhof, Dr HL Palmhert, Sister J Paranzee, Professor OW Prozesky, Professor B Schoub and Sister E Vogel. The National Institute for Water Research provided Figures 1 to 6.

We also wish to acknowledge the very pleasant association and exchange of ideas with our colleagues of the collaborating organizations.

LIST OF TABLES

LIST OF FIGURES

1 SUMMARY

A water reclamation plant was established in Windhoek, SWA/Namibia in 1969 as the only means at the time of augmenting this city's inadequate domestic water supply. This scheme was based on a clearly defined policy of total reclamation strategy and entailed three integrated lines of defence (Henzen and Schutte, 1979). These lines of defence included :

- sewage catchment quality control based on the diversion of industrial discharges containing potential harmful chemical compounds from the domestic sewage collection system;

- efficient reclamation technology backed by vigilant control. Aspects of concern are the efficiency of such a plant to remove pathogenic micro-organisms, toxic metals and organic compounds which may be mutagenic, teratogenic or have other detrimental effects;

- vigilant surveillance of the final water produced which includes a comprehensive determination of the microbial and chemical quality of the water as well as the use of early warning systems, based on biological sensors such as fish;

- the continuous measurement of the chlorine content of the water and also those chemical constituents that might be measured on a continuous basis such as dissolved organic carbon and the ultraviolet absorption of the water.

Since the early nineteen-seventies microbiological and chemical quality control studies had indicated that the product of the reclamation plant was of good quality and conformed to generally accepted drinking-water criteria.

When the reclaimed water was introduced into the municipal water reticulation system, the South African Institute for Medical Research (SAIMR) was requested to undertake a microbiological surveillance programme and, in view of its medical expertise, also to undertake the

analysis and health-orientated interpretation of bacteriological and virological results obtained by it and three collaborating bodies. The latter comprised the National Institute for Water Research (NIWR) of the Council for Scientific and Industrial Research, the Department of Water Affairs (South West Africa/Namibia) and the Windhoek Municipality. In addition, the SAIMR was contracted to expand epidemiological studies to assess the health effects, if any, of reclaimed water consumption on the population of Windhoek.

This report records the results of the studies undertaken by the SAIMR during the decade 1974 to 1983 inclusive. The report first deals with the microbiological surveillance of the reclaimed water and the conclusion is drawn, on the basis of more than 4 000 samples tested, that reclaimed water conformed to generally accepted quality standards laid down for domestic water supplies. From a virological point of view, the source of reclaimed water, i.e. human waste water, was shown to be consistently contaminated with potentially pathogenic viruses, in contrast to the untreated conventional surface water sources. The water was regularly tested at different stages in the reclamation process. Viruses became progressively fewer in number and have been consistently absent from the final stages in the process.

On the basis of the microbiological studies the conclusion is drawn that the reclaimed water is fit for human consumption.

Epidemiological studies were expanded in 1976 and embraced both potential short-term (mainly infectious) and long-term (mainly non-infectious chronic) effects.

The short-term effects considered were the potentially waterborne infectious diseases with particular reference to diarrhoeal diseases and viral hepatitis. Long-term effects included conditions such as cardiovascular disease and malignant neoplasms. Cardiovascular disease has elsewhere been related to water quality, especially 'hardness' of water, while malignant neoplasms have been attributed to the presence of certain carcinogenic chemical substances. This study was carried

out primarily by a method which employed the in-depth documentation of all episodes of the disease concerned in the case of diarrhoeal disease and hepatitis. The mortality studies employed death certificates and hospital records as the main data source. All data were coded and entered in a computerized database.

1.1 Diarrhoeal diseases (DD)

Altogether more than 15 000 episodes of DD were investigated during a continuous period from 1 August 1976 to 31 March 1983. The commonest bacterial causes of DD were Salmonella and Shigella which comprised over 80% of all isolates i.e. about 16% of all cases and the paucity of laboratory isolates underlines the importance of the epidemiological studies. Virus studies yielded insufficient data. DD prevalence was entirely related to socio-economic factors and not to the nature of the water supply. In two socio-economically similar groups of Windhoek residents, those receiving reclaimed water had a consistently equal or lower DD incidence than those receiving water from conventional sources only.

1.2 Viral hepatitis

Almost 1 000 jaundiced patients were investigated during this study. Of these, 282 were investigated utilizing the full battery of both hepatitis A (HAV) and B (HBV) markers. It was shown that 91,1% of blacks and 87,2% of coloureds, all of whom were on the conventional water reticulation system, had hepatitis A in the past, in contrast with only 52,3% of whites, some of whom received reclaimed water. When whites living in reclaimed water areas were compared with whites living in conventional water areas, a total of 17 with acute HA (IgM positive) were recognized in the former and 14 in the latter during the period of study (1980-1982). It was concluded that hepatitis A prevalance in Windhoek was unrelated to water supply but a direct consequence of general environmental conditions and personal hygiene. It was also found that in only 2,2% of all jaundiced black patients, the

jaundice was due to hepatitis A, in contrast with 36,1% of jaundiced white patients. This study supports other workers who have concluded that hepatitis A was a rare cause of jaundice in black Africans.

Hepatitis B, which is not a waterborne infection, was nevertheless studied. This was found to be far more common than is generally appreciated. Almost half of all jaundiced black and coloured patients had evidence of prior infection with HBV whilst the corresponding rate in whites was 19,8%. The bias introduced by studying only jaundiced people was appreciated and the HBV prevalence in the general population may be considerably lower.

1.3 Mortality pattern

A total of 3 000 deaths which occurred in Windhoek was studied. Several thousand additional deaths of persons referred from centres outside Windhoek were excluded on the basis of bias due to selection for serious or unusual diseases and complications. The small size of the Windhoek population (75 000 to 100 000 during the study period) necessitates this study to be continued for cause-specific mortality data to become meaningful. Nevertheless at this stage it is clear that the usual differences related to socio-economic conditions, stress-factors etc., are operative also in Windhoek. More than 60% of all deaths in whites were due exclusively to cardiovascular disease and malignant neoplasms. The leading cause of death in blacks and coloureds was diarrhoeal disease with tuberculosis as another important cause. Cancer of the lung was the commonest of all malignancies in all population groups and was only slightly exceeded by carcinoma of the oesophagus in blacks. No attempt has been made to relate mortality to water supply and it is our opinion at this stage that it is highly unlikely that this will be possible within the foreseeable future. On the other hand, sudden unrecognized defects in water supply or in other environmental factors giving rise to chronic tissue damage may well first reveal themselves through a marked alteration in the mortality pattern. If for this reason alone, the mortality study must be continued.

In conclusion, the authors of this report are of the opinion that within the limits of the epidemiological studies done, no adverse effects on health attributable to the consumption of reclaimed water could be demonstrated. This conclusion is further supported by the study on the health effects of the indirect reuse of reclaimed water by the County Sanitation Districts of Los Angeles County in Whittier, California (Nellor, Baird and Smyth, 1984). They concluded that no viruses could be detected in chlorinated reclaimed water. An evaluation of health and vital statistics over a period of 12 years showed that residents of the area that received reclaimed water experienced no increased rates of infectious diseases, congenital malformations, infant and neonatal mortality, low birth weight, cancer incidence or deaths due to heart disease, stroke, cancers of the stomach, rectum, bladder or colon, or all cancers combined, when compared to residents of two control areas that did not receive reclaimed water.

2 INTRODUCTION

Southern Africa is a region with restricted water resources and this is even more true for the arid South West Africa/Namibia. It is almost three decades ago that the City of Windhoek realised that the planned expansion of their water resources would not be able to meet crisis situations such as for instance a severe and prolonged drought. Following further studies it was soon realised that the city's water supply could be augmented by the reclamation of secondary treated sewage effluents. However, it had to be shown that this reclaimed water would not introduce a health hazard, if it were to be used as a potable water source.

The City of Windhoek therefore commissioned the National Institute for Water Research (NIWR) of the Council for Scientific and Industrial Research (CSIR) to develop the necessary expertise to reclaim water for unrestricted reuse. Early in 1968 a critical shortage in water supply was expected and the need for a full-scale reclamation plant became urgent. Such a plant was commissioned during January 1969 and its design and operation was based on the results of a pilot plant operated by the NIWR in Windhoek over the period 1964 to 1968.

The Water Research Commission was established during 1971 and immediately embarked on an ambitious research programme to expedite the development of the technology of water reclamation and to study the health effects of such water. A research/demonstration facility was constructed by the CSIR at Daspoort in Pretoria and the Windhoek plant was modified from time to time to incorporate advantages gained from the latest research results with financial assistance of the Water Research Commission.

The first well documented study to show the relationship between the quality of a potable water and the health of the consumer was based on a study in 1855 that a cholera epidemic in London could be related to polluted drinking water. Since that time the question as to how and in what way potable water affects the health of man remains. This difficult question to answer is due to the fact that man is also exposed to a number of other factors operative in his environment and

that the interaction of these factors is difficult to detect and quantify. Ideally, studies to determine such effects should be carried out on man himself. However, this cannot be done for a multitude of reasons. Therefore epidemiological studies to determine the relationship between the quality of drinking water and the health of the consumer is an attractive and perhaps only way to obtain the answers to this vexed problem. Such a study can be based on two well-defined approaches i.e. the determination of the concentration of the chemical and biological constituents known to cause acute diseases in man and the biological assessment of the effect of potable water on the consumer. The latter study is normally based on the use of sensor organisms such as fish, protozoa, cell and bacterial cultures. In this way a well-defined protocol exists and is continuously improved to study the effect of water on the consumer.

The first line of defence i.e. a knowledge of the concentration of various constituents in water will however not necessarily give information on the possible long-term effects on man and must therefore be followed by epidemiological studies of the population exposed to the water. Therefore a close and continuous surveillance must be maintained on the health of the population. This approach is common to that followed in the development and production of medicines and provides a mechanism to protect the health of the consumer or to raise an alarm should problems be observed.

Impetus to epidemiological studies in the field of water supply was given with the advent of direct water reclamation for potable reuse and the results of a study carried out on a community deriving their water supply from the Mississippi River (Harris, 1974). Since then a number of epidemiological studies have been reported and were reviewed during 1980 by a committee of the United States National Academy of Sciences (NAS). They concluded from their review (12 epidemiological studies) that the studies failed either to support or to refute the results of positive animal bio-assays suggesting that certain chemicals such as the trihalomethanes may cause cancer in humans. References to the epidemiological studies are given in Section 8.3. The reason for the inconclusive results from these epidemiological studies is perhaps the difficulty of identifying small effects in a

population (NAS, 1980). The complexities introduced when humans are exposed to multiple contaminants in low concentrations (ug/ℓ or less) in drinking water, make it virtually impossible to establish a causal link between water quality and the health of the consumer.

In spite of these difficulties, a study group of the World Health Organization called in 1975 for the following action: "When there is a plan or project for a water reclamation scheme in any area, an immediate basic study of the general health, prevailing diseases and mortalities should be carried out on the population. This should be continued until commissioning of the plant and then extended into the future in order to observe any statistically significant differences."

For the reasons outlined above, the Water Research Commission entered into an agreement with the South African Institute for Medical Research (SAIMR) to study both the microbiological quality of the reclaimed water and the epidemiology of the introduction of this water to the community of Windhoek. This report then provides a summary of the research carried out over a decade by the SAIMR in collaboration with the Water Research Commission.

3 THE DEVELOPMENT OF THE TECHNOLOGY TO RECLAIM POTABLE WATER FROM TREATED SEWAGE EFFLUENT

In 1957 the Goreangab Dam and Water Purification Works were being constructed to supplement the existing water supplies derived from the Avis Dam and boreholes. The new Gammams Sewage Works comprised biological filters and a series of maturation ponds and was commissioned in 1960.

The domestic supply to Windhoek therefore consisted of two surface impoundments (Avis and Goreangab) together with 36 boreholes, scattered throughout the city. The purified water was pumped to main distribution reservoirs from where it was mixed with the water pumped directly from the boreholes.

During 1972 a further source of potable water to Windhoek became available when the Department of Water Affairs commissioned their Von Bach Dam on the Swakop River. The Von Bach water was purified at the

dam and then pumped to a receiving reservoir at Windhoek from where it was released to the reservoirs operated by the City of Windhoek.

The reclamation plant, commissioned in 1969, was designed to treat 4 500 m^3/d and comprised algal flotation, foam fractionation, disinfection and activated carbon treatment (Figure 1). The integration of the reclamation plant with the Goreangab Dam Waterworks is shown in Figure 2.

The reclamation plant was modified to incorporate research benefits obtained from Daspoort (Pretoria) which resulted in the omission of the algal flotation tank and foam fractionation (Figure 3). These treatment stages were replaced with the high pH lime treatment and ammonia stripping processes. A flow diagram of the reclamation plant, showing the process stages together with the sampling points (designated WR) is shown in Figure 4.

Additional purification capacity at the Gammams Sewage Works was provided by the introduction of an activated sludge plant. An effluent low in ammonia-nitrogen was now available and this led to the omission of the high pH lime treatment and the ammonia stripping process. A flow diagram of the existing plant is shown in Figure 5. The effluent from the biofilter plant is treated separately from that of the activated sludge plant in three maturation ponds (numbers 1, 10 and 11, Figure 6). The other maturation ponds (numbered 2 to 9, Figure 6) are used for further treatment of the activated sludge plant effluent prior to reclamation. The integration of the reclamation plant with the sewage treatment plant is also shown in Figure 6. The integration of the reclamation plant with the Goreangab Dam Water Treatment plant remains as shown in Figure 2.

4 THE DEVELOPMENT OF A RESEARCH AGREEMENT BETWEEN THE WATER RESEARCH COMMISSION AND THE SAIMR

The urgency to provide Windhoek with an additional source of water did not allow enough time to conduct a thorough epidemiological survey of the prevailing disease pattern in Windhoek. There was little doubt that the bacteriological water quality would be of a high standard but a number of unanswered questions remained. It was also reasoned that to ensure confidence, more than one organisation should independently

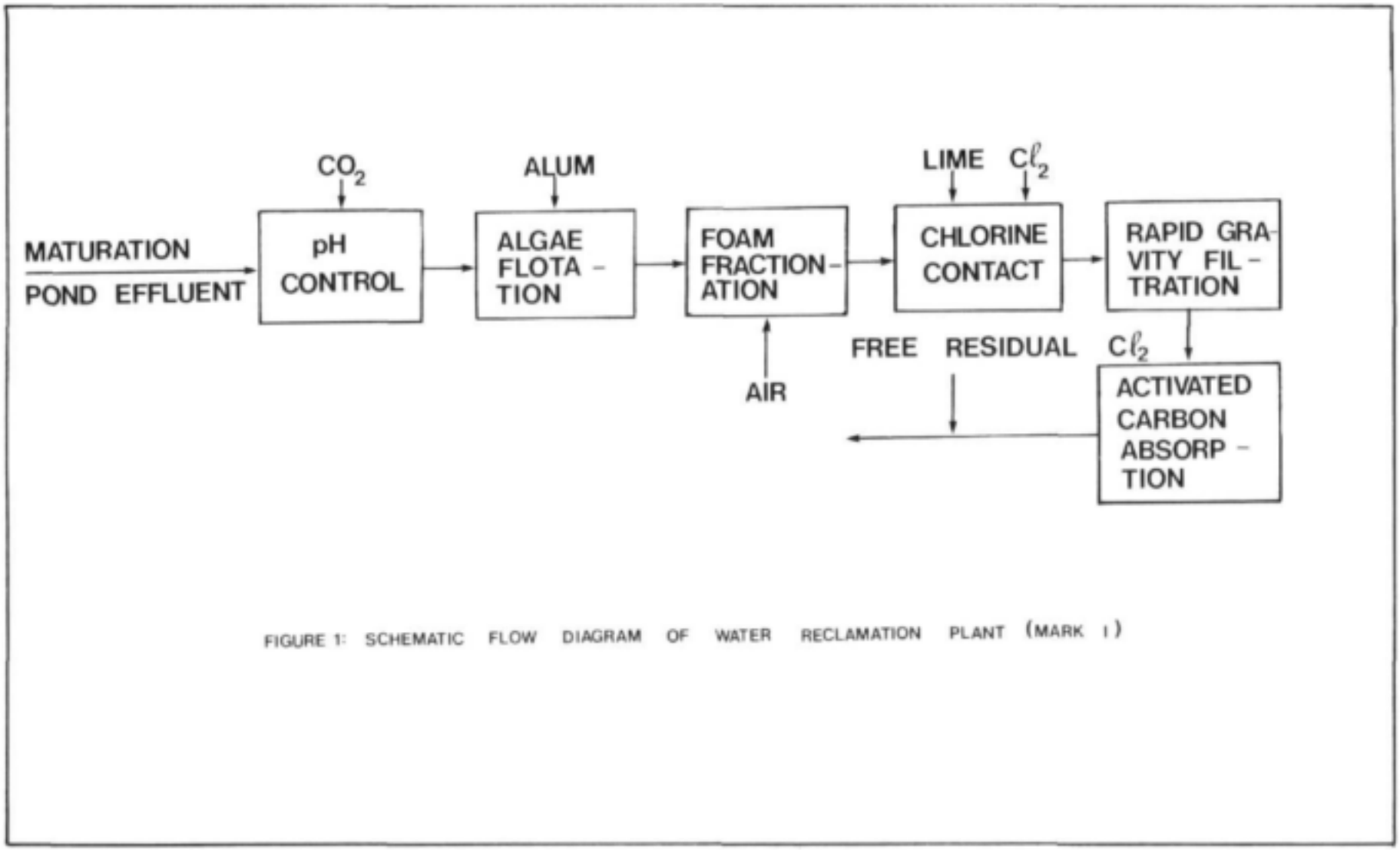

FIGURE 1: SCHEMATIC FLOW DIAGRAM OF WATER RECLAMATION PLANT (MARK I)

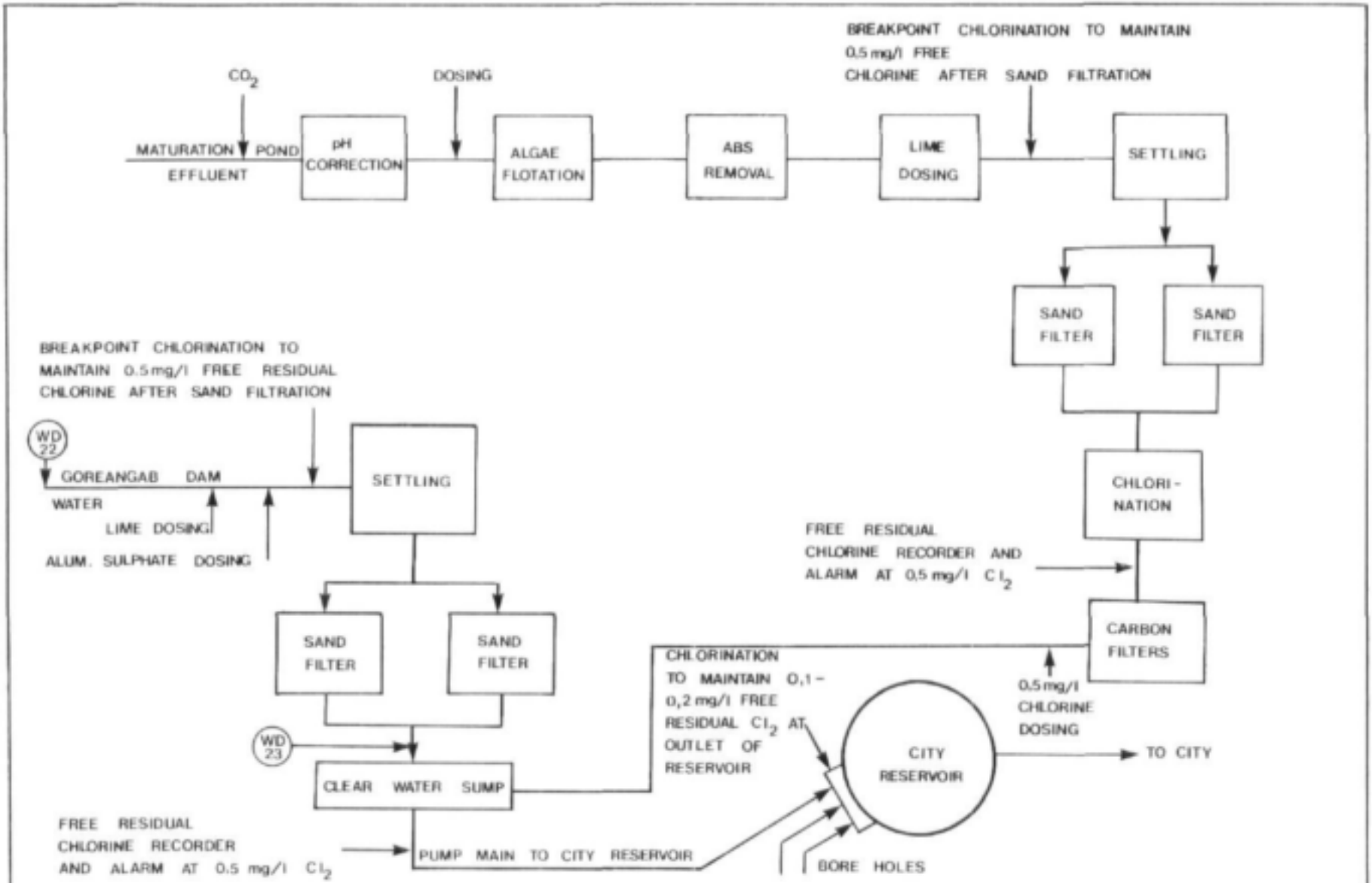

FIGURE 2: INTEGRATION OF GOREANGAB DAM AND RECLAIMED WATER PURIFICATION PLANTS.

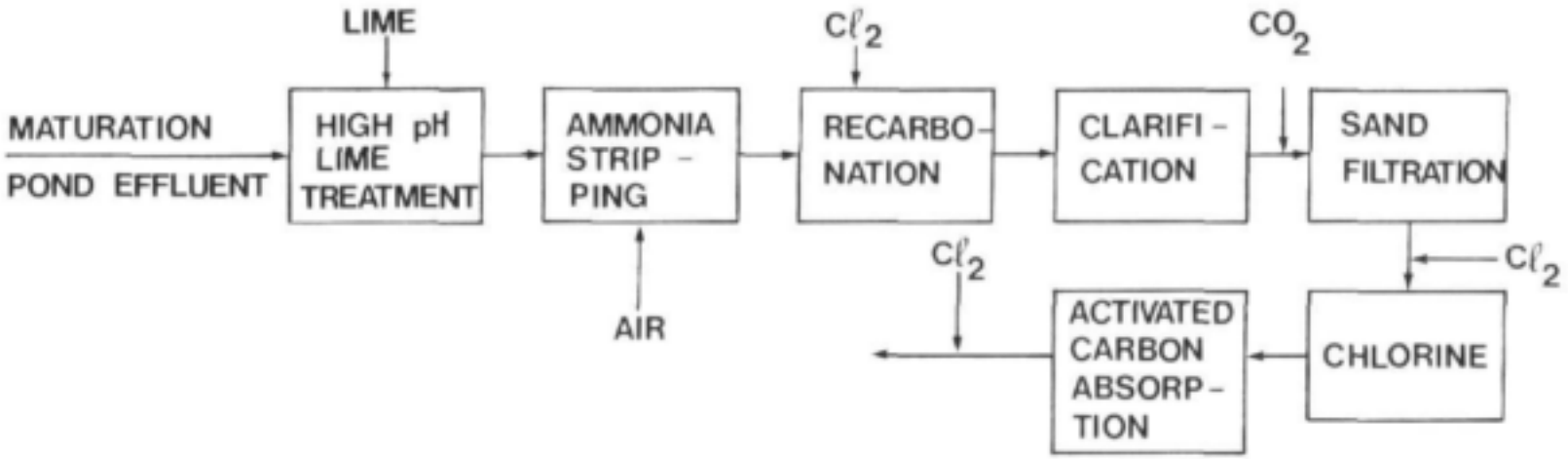

FIGURE 3: FLOW DIAGRAM OF WATER RECLAMATION PLANT (MARK II)

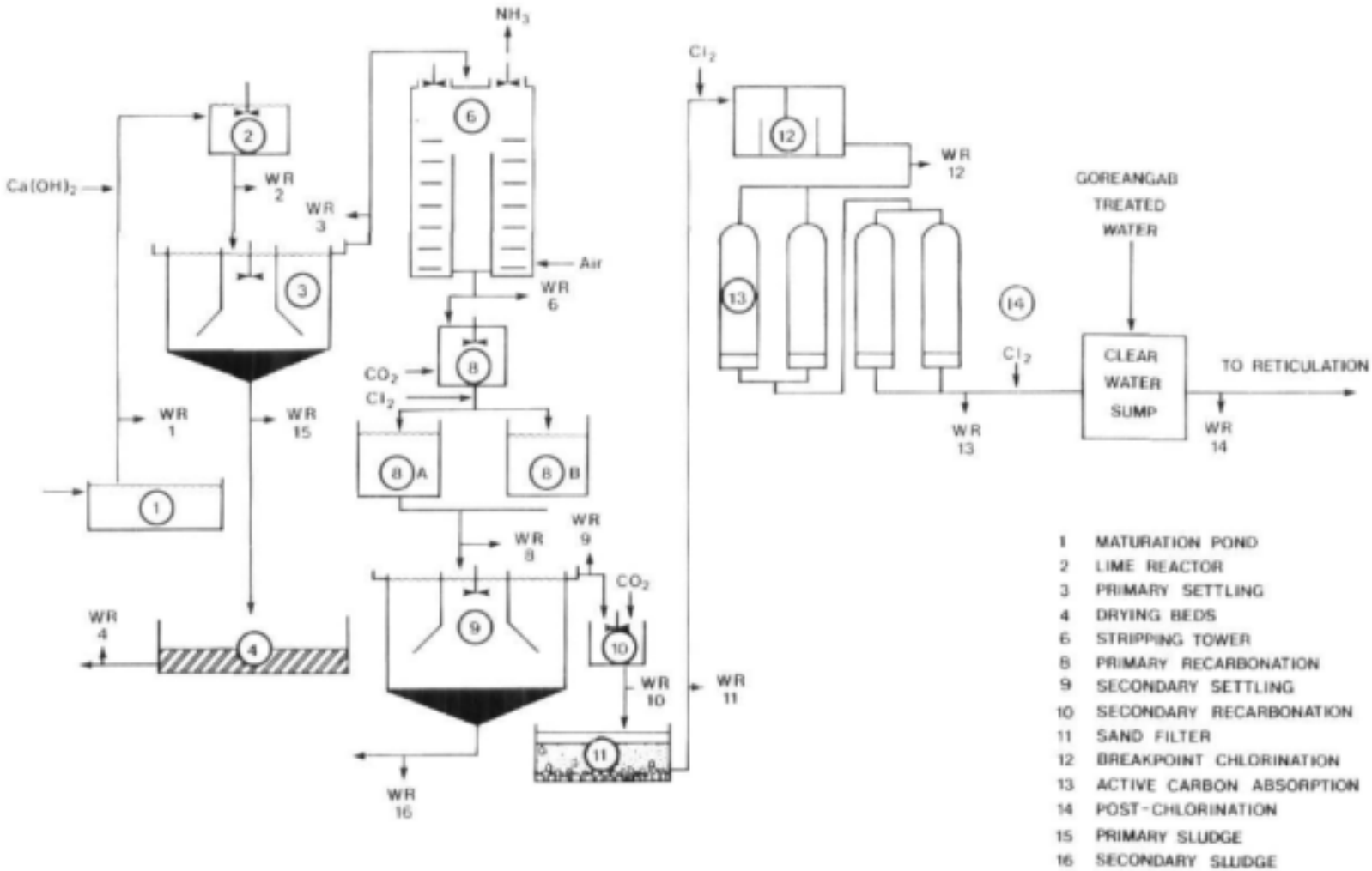

FIG 4 : FLOW DIAGRAM OF MODIFIED WINDHOEK WATER RECLAMATION PLANT (MARK II)

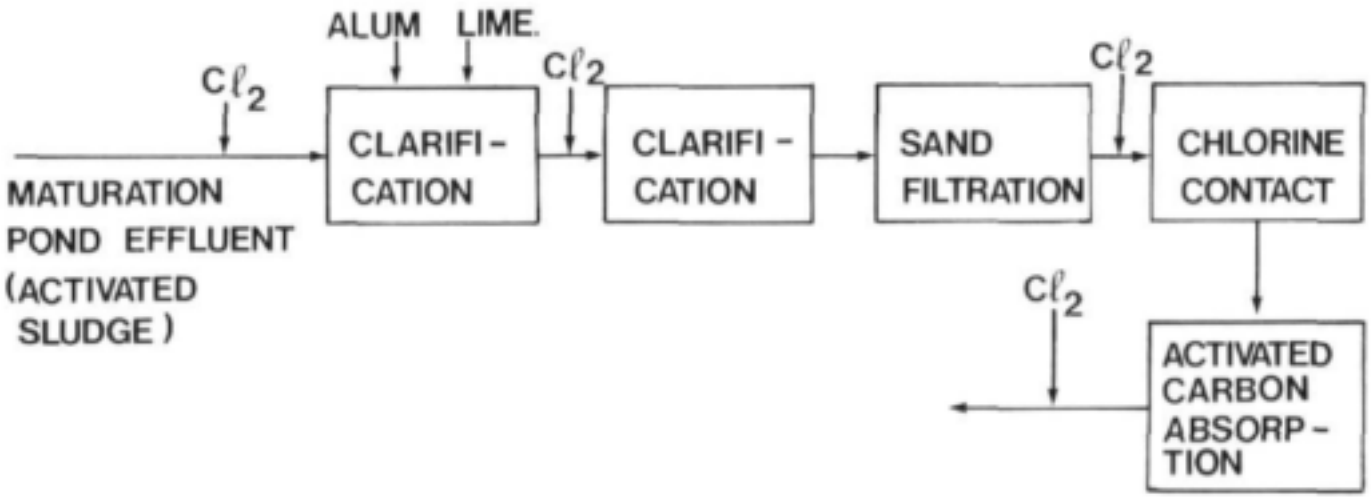

FIGURE 5: FLOW DIAGRAM OF WATER RECLAMATION PLANT (MARK III)

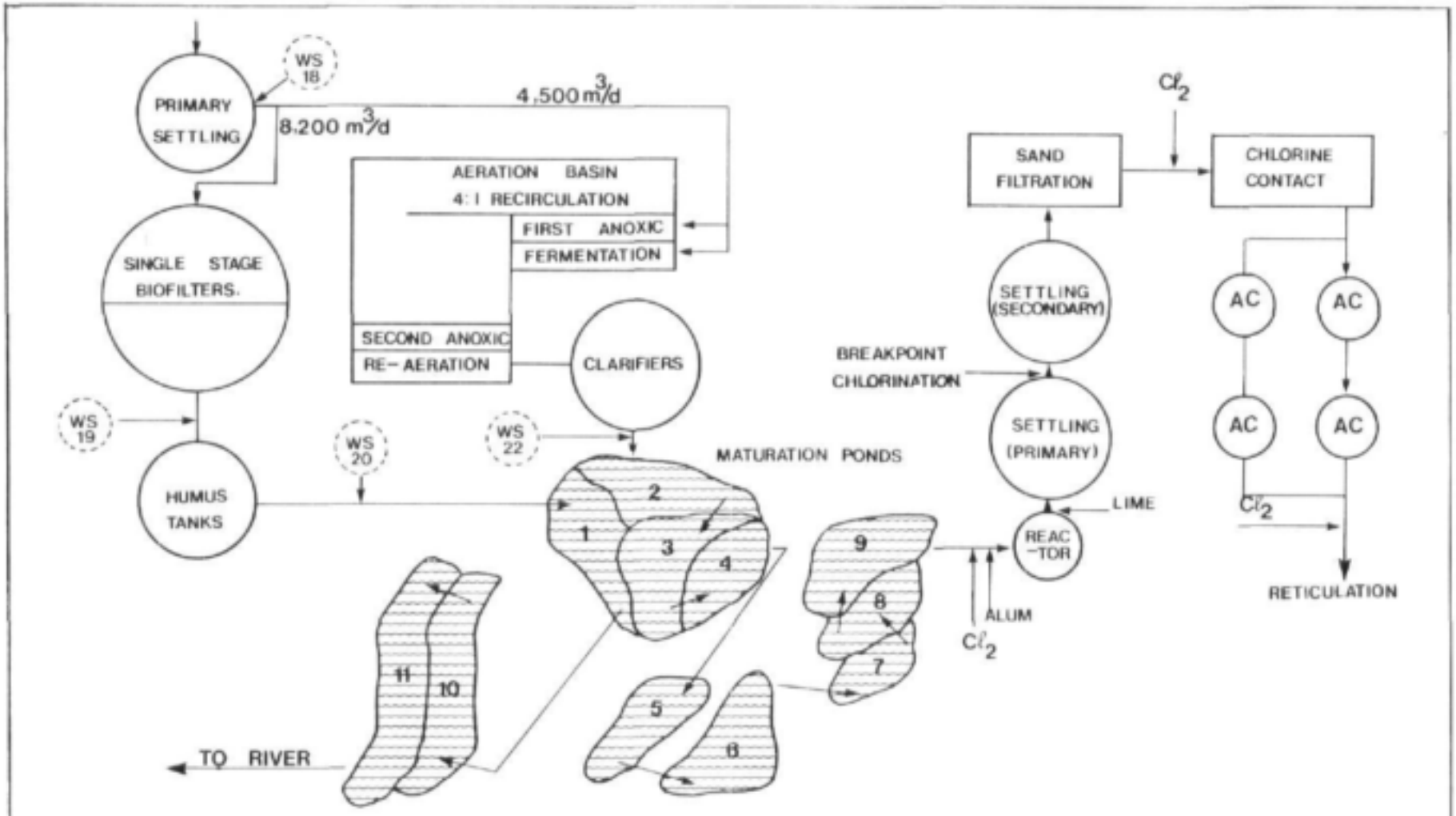

FIGURE 6: FLOW DIAGRAM OF THE WINDHOEK SEWAGE TREATMENT AND RECLAMATION WORKS (MARK III)

study the quality of the water produced. The NIWR, Windhoek Municipality and the Department of Water Affairs analysed the water at regular intervals but none of these organisations had the necessary medical background to evaluate the health implications of the consumption of reclaimed water. Therefore the Water Research Commission entered into a contract with the CSIR, through its NIWR, the Municipality of Windhoek and the SAIMR during 1973.

The SAIMR was requested to participate in the surveillance testing of the virological and bacteriological quality of the different sources of potable water as supplied in Windhoek and also to initiate an epidemiological study of the Windhoek population. This study consisted of the following tests :

(i) A weekly testing for the presence of human enteric viruses in 10-litre samples of the raw water fed to the reclamation plant (purified sewage effluent) as well as the finally treated reclaimed water.

(ii) A spot checking (at least monthly) of the settled sewage to the Gammams Sewage Purification Works, the humus tank effluent, the maturation pond effluent, the reclaimed water after different stages of treatment, the water after different stages of purification in the Goreangab Water Treatment Plant, the water from the boreholes and the treated water from the Von Bach Dam for total coliforms, Escherichia coli, Pseudomonas aeruginosa and coagulase positive staphylococci.

(iii) Epidemiological studies supported by laboratory tests for viral infections and a data bank of the prevailing disease pattern of the Windhoek community.

The above agreement was replaced by a more comprehensive agreement between the Water Research Commission and the SAIMR during 1975. This contract was again modified during 1977 to further broaden the scope of the studies and made provision for four separate bodies (viz. the NIWR, Windhoek Municipality, SAIMR and the SWA/Namibian Department of

Water Affairs) to collect more extensive data on the quality of drinking water supplied to the City of Windhoek. The data so collected were submitted to the SAIMR who in turn prepared annual reports, based on the data and submitted these to :

(i) the steering committee meetings for consideration;
(ii) the health authority in Windhoek.

5 METHODS, STUDY POPULATION AND MATERIALS

In order to standardise the analytical methods used and the sampling points and frequency of sampling, a subcommittee was appointed to clarify these matters. This subcommittee was chaired by the NIWR and consisted of representatives of all the research workers connected with this project. This procedure was thought necessary to ascertain that an independent surveillance of the water quality could be maintained and also that the frequency of sampling would be such that daily observations could be made. In addition, the SAIMR collected data on the prevailing disease pattern of the community in Windhoek.

5.1 Sampling points

The letter W was used to designate samples from Windhoek and the second letter designated sewage, reclaimed water or dam water, whichever was applicable. The codes WD (Goreangab Dam water), WS (Gammams Sewage Works) and WR (reclamation plant) were accordingly assigned to the sampling points, the locations of which were as follows :

(a) Reclamation plant : (Figure 4)

WR 1 Raw water intake and equivalent to effluent from maturation pond No 9 (Figure 6).
WR 2 High pH lime treatment
WR 3 Primary settling
WR 4 Effluent after drying beds
WR 6 Ammonia stripping
WR 8 Primary recarbonation
WR 10 Secondary recarbonation

WR 11 Sand filtration
WR 12 Breakpoint chlorination
WR 13 Activated carbon filtration
WR 14 Final water

(b) Sewage works : (Figure 6)

WS 18 Settled sewage
WS 19 Biofilter effluent
WS 20 Humus tank effluent
WS 22 Activated sludge effluent

(c) Water treatment plant : (Figure 2)

WD 22 Goreangab Dam : raw water
WD 23 Goreangab Dam : treated water

(d) Additional samples

WD 24 Von Bach Dam treated water at the reservoir in Windhoek
WD 25 Avis Dam : raw water
WD 26 Avis Dam : treated water
WD 27 Pahl Quelle group of boreholes
WD 28 Kleine Kuppe group of boreholes

Samples were collected daily, seven days a week, but not necessarily from all the sampling points at the same time. Sampling frequency also varied according to needs. For example, modification to a particular treatment process would be followed by an increased frequency of tests on the affected stages.

The reclamation plant was operated intermittently as and when the need for augmentation of water supplies arose. This provided an unique opportunity to study the possible health effects, if any, of the reclaimed water.

5.2 Bacteriological counts

Initially, the tests done consisted of a total plate count, coliforms

and E. coli on all samples, supplemented by tests for the presence of Clostridium perfringens, Pseudomonas aeruginosa, enterococci and Staphylococcus aureus in treated water samples.

The methodology for these tests was standardized by the Technical Subcommittee and is outlined below :

- Standard plate count
  The medium used was Oxoid yeast extract agar. Four plates were prepared for each dilution and were incubated at 37°C for 48 h.

- Clostridium perfringens
  These were isolated by means of the membrane filter technique on Wilson-and-Blair culture medium. Identification was done by Nagler reaction.

- Staphylococcus aureus
  The membrane filter technique with Difco mannitol agar was used. The cultures were incubated at 37°C for 48 h and their identity confirmed by DNase production on DNA agar plates. The number of colonies to be tested by this method was calculated in the manner as described under E. coli above.

- Pseudomonas aeruginosa
  A qualitative membrane filter technique with Drake's culture medium was used on 100 mℓ samples in triplicate in order to test a total of 300 mℓ. The additional use of acetamide medium served to indicate the presence of presumptive Pseudomonas aeruginosa when a characteristic green colour developed.

- Enterococci
  The membrane filter technique and Difco M enterococcus agar were used. The filters were incubated on agar at 45°C for 48 h. Confirmatory evidence was obtained by the use of 0,4 g potassium tellurite/kg agar.

  These methods are detailed by Grabow and Isaäcson (1978), Grabow and Du Preez (1979) and Grabow (1981, Appendix 9.1).

- Coliforms and E. coli

  The membrane filter technique was applied using disposable Gelman filters. MacConkey membrane agar was used for culture. E. coli was identified by indole production at 44°C. If less than 20 colony-forming units (CFU) were obtained, each colony was tested, and when more than 20 CFU were obtained, 20 CFU were selected and tested.

- Vibrio cholerae

  The method of isolation was described by Isaäcson (1975) and involved enrichment in alkaline peptone water and the selective TCBS agar containing thiosulphate, citrate, bile salts and sucrose.

5.3 Human enteric virus counts

Counts for human enteric viruses were done on 10-litre samples of treated waters and on 1-litre samples of sewage effluents. Sewage samples were collected weekly at points WS 18 and WS 20 and the other fortnightly at points WR 1, WR 12, WR 14, WD 22 and WD 23. The Amicon filtration and cell culture techniques were used for virus isolation. Cultures showing evidence of viral multiplication were forwarded to the National Institute for Virology for identification. Quantitative tests were done on the WS samples and the results expressed as $TCID_{50}$ (50% tissue culture infectious dose). On the WR and WD samples only qualitative tests (virus present or absent) were carried out.

5.4 Epidemiological studies

Epidemiological studies which had been commenced in the early nineteen-seventies were expanded during 1976. It was generally accepted that studies to assess the health effects of a water supply on a population was an exceedingly difficult undertaking. There are many reasons why this should be so but the most important one is, without doubt, the presence of a large number of confounding variables. This was appreciated by us as early as 1973 and we were not surprised therefore by the frequency with which this problem has presented itself.

The accomplished fact of an established reclaimed water supply to the city of Windhoek, critically in need of water augmentation, presented the opportunity to carry out epidemiological studies to assess both the short-term and long-term effects on health, if any.

In addition it was recognized that such an undertaking would also provide opportunities for studies, the results of which, though not directly related to reclaimed water consumption, would furnish a database on the health profile of the population, useful for future water related and other research projects.

The following long-term investigations were planned and carried out :

- diarrhoeal diseases surveillance (DDS);
- investigation of the incidence and the aetiology of jaundice;
- mortality profile in SWA/Namibia.

The studies were mainly based on the collection of certain morbidity and mortality data, the former backed by laboratory tests. Data collection was carried out by nursing sisters. Standard computer coded forms were designed and used for this purpose. Appendix 9 contains two basic blank data forms as used for morbidity and mortality studies respectively.

5.5 Demographic data

The Town Planning section of the Windhoek City Engineer's Department kindly provided all demographic data which included the age, sex and race structure of the total population of Windhoek using 5 year age intervals, projected up to the year 2 000 (Demasius, 1976). The demographic data for specific major areas were only available for the census year (1975), using broad age categories (0 - 19, 20 - 39, 40+). These categories were unsuitable for calculating age - specific rates.

The 1981 census data for specific areas were also found to be unsuitable for similar reasons (age structure, insufficiently defined) and in addition the census tracts of major areas were different from those

used in the 1975 census.

Since 1976 close collaboration with that Department was maintained in order to achieve as high a standard of accuracy as possible in the epidemiological analyses. Windhoek, according to the 1975 census, had a total population of some 75 000 persons of which just over 32 000 (44%) were white, 33 000 (44%) black and 10 000 (12%) coloured.

Use was made of the 1975 Windhoek tract for the purpose of charting and collating the study data. Until recently the population was residentially segregated according to ethnic groups. Reclaimed water was supplied only to part of the white population according to the water reticulation system. When reclaimed water was supplied, it was mixed with water from conventional sources. Augmentation by reclaimed water was practiced only when needed; more often than not the residents in the reclaimed water distribution areas received water from conventional sources only. This provided for a comparison of the health statistics of reclaimed water recipients with those of conventional water recipients, and also for a vertical comparison of data obtained on a sector of the population during reclaimed water consumption and during conventional water consumption. Additional control data were provided by the black and coloured populations in which general standards of sanitation and hygiene are considerably lower than in whites, but whose conventional water supply comes from the same sources and is not augmented with reclaimed water.

Figure 7 depicts a diagrammatic map of Windhoek showing the 'major census areas' and their numerical codes. Area 23 is the black residential area Katutura, and area 22 is the coloured residential area Khomasdal. The remainder comprises the central business district and white residential areas. The sector enclosed by a thick black line indicates those white residential areas and the central business district to which reclaimed water is supplied when required. The map is a reproduction of a large working map on which numerous small 'enumeration areas' are also shown and on which the reclaimed water supply area is more accurately depicted than was possible here.

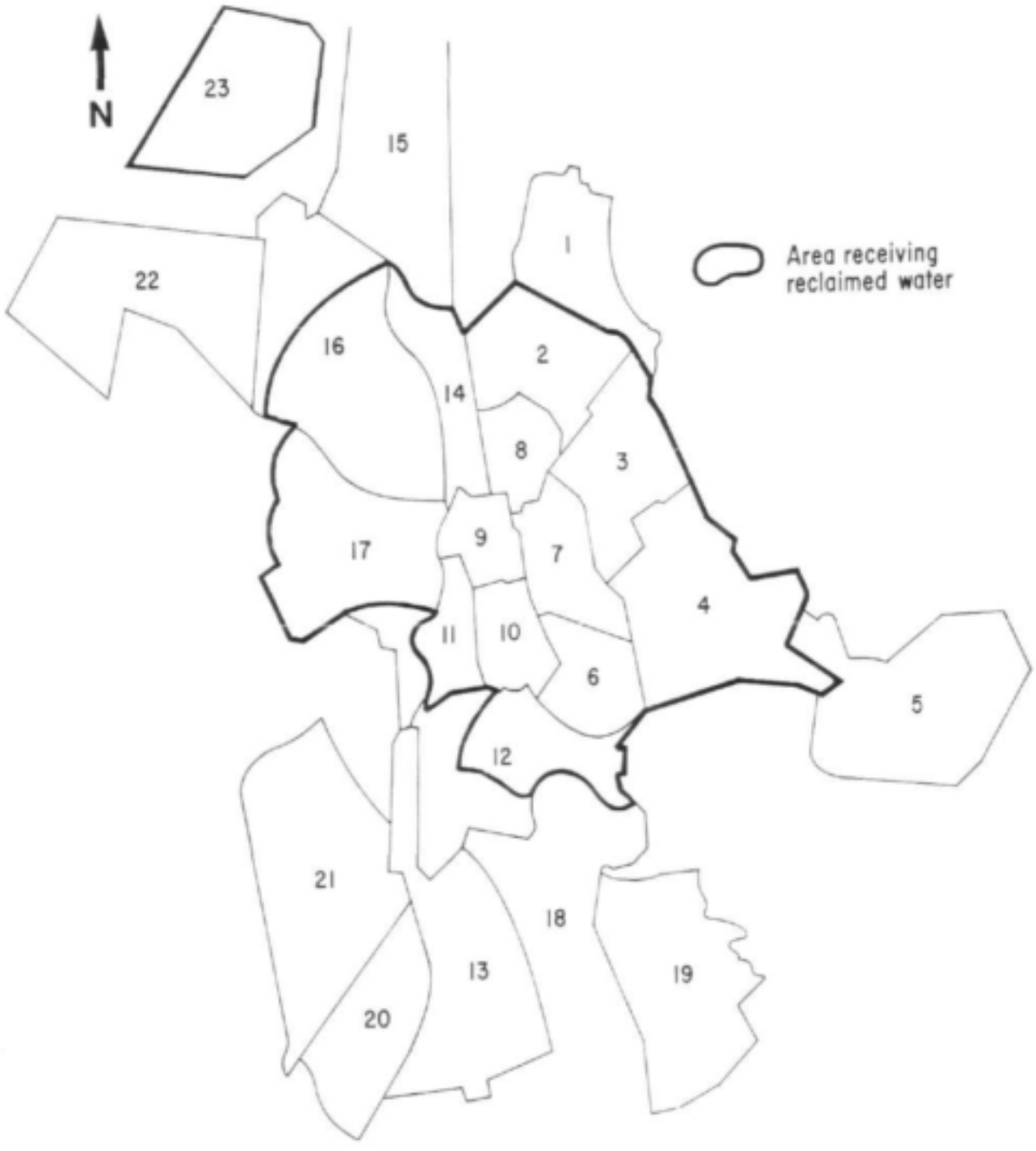

FIGURE 7: MAP OF WINDHOEK AS PREPARED BY THE MUNICIPALITY AND SHOWING THE MAJOR AREAS DELINEATED FOR THE 1975 POPULATION CENSUS

5.6 Statistical methodology

All coded data collected since 1976 were entered onto a computerized database. Projected populations, based on the 1975 census figures, were used to establish rates for each area under study as defined by major area codes.

Due to insufficiently defined age-specific denominator data for specific areas, incidence rates of diarrhoeal disease for children less than 15 years were calculated using the total population of the respective areas as denominators. The chi-square test of independence was performed to test the null hypothesis that the two criteria of classification, i.e. the area under study (reclaimed versus conventional) and the presence or absence of the disease under study were independent.

The small size of the Windhoek population (ranging from approximately 75 000 to 100 000 during the study period) yielded low numbers of deaths, when classified by cause, hence cause-specific proportional mortality rates of the total mortality by race were calculated. These rates have the obvious disadvantage of mutually influencing each other but were nevertheless preferable to the use of total population denominators (crude rates).

5.7 Diarrhoeal diseases surveillance (DDS)

The study included every patient with diarrhoea who gave a Windhoek address and who attended a hospital, clinic or general practitioner's consulting rooms. There are relatively few private practitioners in Windhoek and their collaboration was readily obtained.

It was recognized from the start that this system selected only for study subjects who felt sufficiently ill to seek treatment, but the realities of the situation made this the most practical and economic approach. A nurse epidemiologist completed the data form. Stool or rectal swabs were cultured for *Salmonella*, *Shigella* and *E. coli*. The SAIMR provides the only medical laboratory service to the territory,

therefore laboratory investigations carried out during the study were standard and in accordance with procedures laid down in the SAIMR Laboratory Manual.

Cultures for Campylobacter, Yersinia enterocolitica and Vibrio were not routine investigations during most of the duration of the DDS project and were therefore not included. Vibrio cholerae emerged in disseminated epidemic form in Southern Africa only during 1980 and tests for this organism were subsequently introduced in Windhoek as part of the routine battery of bacteriological tests on stools and rectal swabs.

Isolates of Salmonella, Shigella and E. coli were forwarded to the Salmonella Reference Laboratory in Johannesburg where salmonellas and shigellas were serotyped and, in cases of S. typhi, phagetyped. E. coli was serotyped to determine its enteropathogenicity. Toxigenicity tests which are now standard procedure for E. coli were not available during the greater part of the study. The results of the various tests were computer coded (Appendix 9.4).

5.8 Hepatitis studies

All jaundiced patients presenting at hospitals, clinics or private consulting rooms were, in principle, admitted to the study, but not all were completely investigated. Personal data were collected and coded for computer analysis. Clinical and laboratory data were also obtained. Laboratory tests changed over the years in that they became automated, and international uniformity in nomenclature and measurements were adopted. For the purpose of this report the earlier records using the previous nomenclature and units of measurements have been converted to the SI units currently in use. Tests of hepatic and other functions carried out on all patients were as follows : (see also Appendix 9.5):

Name of test	Reference value
Bilirubin - total	4-21 µmol/ℓ
Bilirubin - direct	0-4 µmol/ℓ
Total serum protein	55-80 g/ℓ

Serum Albumin	30-55	g/ℓ
γ- Glutamyltransferase (GGT)	10-50	U/ℓ (males)
	7-32	U/ℓ (females)
Alkaline phosphatase (ALP)	36-92	U/ℓ
Aspartate transaminase (AST)	10-30	U/ℓ
Alanine transaminase (ALT)	6-37	U/ℓ

From 1976 to 1980 the HBs antigen test was the only specific viral hepatitis B (HB) test available and this was done in Windhoek on sera of 327 patients by means of counter-current electrophoresis until mid-1977 and from then onwards until 1980 by commercially available haemagglutination kit test (Hepanosticon). Subsequently technology was developed to detect specific viral hepatitis A ('infectious hepatitis') antibodies and viral hepatitis B ('serum hepatitis') antigens and antibodies. In this investigation the full battery of new tests was substituted for the old test from 1980 onwards. These tests were carried out by solid phase bead radio-immunoassay (Abbott, North Chicago) at the National Institute for Virology. Interest in hepatitis B, which is not a water-related infection, arose from the fact that no basic data on this disease were available for a SWA/Namibian urban community.

5.9 Mortality data

For reasons of economy and convenience, mortality data were obtained from death certificates. The latter are generally considered unreliable. Nevertheless, they are still recognized to provide useful information within their limitations. This was confirmed recently by Tyrrell and Lloyd (1984) in Scotland for lung cancer.

There were certain advantages peculiar to Windhoek. These included its geographic isolation and, during the earlier years, stability of the population. Windhoek is not highly industrialized and therefore gross industrial pollution of water sources was not believed to greatly impinge on this study.

Windhoek acts as a medical referral centre and therefore many deaths, at least half of the total, occurred in patients referred from other parts of the country. All of these data were collected but were not

analysed for this report. The causes of deaths in referred patients would have introduced bias since referral occurs mostly where patients have diseases or complications for the treatment of which facilities and expertise are not available in their own areas, thus selecting for certain diseases and not others. The standard mortality data form (Appendix 9.3) was basically similar to that used for the DDS. The main difference consisted of a further stratification of blacks by tribes as it has been suggested that variances between tribal customs may manifest themselves in the mortality pattern.

5.10 Other analytical studies

A comprehensive analytical programme comprising both chemical and microbiological tests was instituted and maintained since 1969. The chemical quality of the water does not form part of this report but has been extensively covered in other reports and publications. The results of these studies will be further discussed in Section 7. Therefore only the microbiological quality and the epidemiological studies conducted by the SAIMR are now further discussed.

6 RESULTS AND DISCUSSION

6.1 Virological studies

The SAIMR was not equipped to carry out virological studies in the initial phases of the study. Appropriate laboratory facilities became available in Windhoek only after 1976. Therefore results for virus isolations cover only the period July 1976 to the end of April 1982. During this period a total of 899 virus isolations were made. The results are summarised in Table 1.

The irregularity in the testing of reclaimed water was due to the fact that the plant was operated intermittently as and when augmentation of the water supply was necessary.

Details of the periods of operation and the composition of the water supplied are outlined in Table 2.

Table 1

IDENTITY AND FREQUENCY OF VIRUS ISOLATIONS FROM PROGRESSIVE SAMPLING POINTS DURING WATER RECLAMATION AND FROM TREATED AND UNTREATED CONVENTIONAL WATER SOURCES DURING THE PERIOD JULY 1976 TO APRIL 1982

No. of samples tested	Sewage				Reclamation process						Conventional				Total
	WS 18		WS 20		WR 1		WR 12		WR 14		WD 22		WD 23		
Virus isolated	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Negative	1	0,3	5	6,3	95	60,9	55	100	76	100	109	100	105	100	446
Reovirus	54	16,9	31	39,3	21	13,5									106
Enterovirus															
Polio	64	20,1	0		5	3,2									69
Coxsackie A	1	0,3	2	2,5	0										3
Coxsackie B	36	11,3	8	10,1	13	8,3									57
Echo	63	19,7	10	12,7	12	7,7									85
Mixed	28	8,8	1	1,3	2	1,3									31
Unspecified	19	6,0	2	2,5	2	1,3									23
Mixed reo/enterovirus	25	7,8	3	3,8	3	1,9									31
Unidentified virus	28	8,8	17	21,5	3	1,9									48
Total	319		79		156		55		76		109		105		899

WS 18 = Settled sewage
WS 20 = Humus tank effluent
WR 1 = Reclamation plant raw water intake
WR 12 = After breakpoint chlorination
WR 14 = Final reclaimed water
WD 22 = Raw dam water
WD 23 = Treated dam water

Table 2

THE PERIODS DURING WHICH WATER WAS RECLAIMED AND THE PERCENTAGE BLEND WITH WATER FROM CONVENTIONAL SOURCES

Year	Number of months reclaimed	Percentage reclaimed water in blend		Volume reclaimed	Overall supply
		Average	Range	$x10^3$ m^3	$x10^3$ m^3
1968	3	12,3	3,6 - 20,6	224,7	1 834,2
1969	11	15,1	0,6 - 22,7	889,0	5 890,8
1970	10	15,1	8,8 - 27,7	795,7	5 284,3
1971	3	15,1	0,5 - 24,5	223,3	1 551,4
1972	2	6,6	6,2 - 6,9	53,1	1 060,0
1973	5	15,1	1,6 - 32,8	264,8	3 507,0
1974	1	8,6	-	27,0	544,0
1975	NIL	-	-	-	-
1976	1	1,5	-	10,1	1 131,7
1977	8	15,3	3,3 - 31,5	530,3	6 404,6
1978	3	23,8	9,2 - 41,1	227,7	2 021,4
1979	NIL	-	-	-	-
1980	10	12,9	2,9 - 27,4	527,2	9 484,5
1981	11	22,6	0,9 - 42,0	860,5	8 325,9
1982	12	28,3	7,2 - 40,7	1 183,2	8 617,8
1983	6	24,3	17,7 - 31,9	534,7	4 969,7
1984	7	23,5	2,9 - 36,6	562,5	6 798,6

During critical conditions blended drinking water contained as much as 50% reclaimed water albeit for short periods - less than 3 weeks. The average exposure to reclaimed water over the period of 17 years was 4,7%.

Viruses were isolated only from sewage samples (WS-series) and from WR 1 (maturation pond effluent). Samples obtained after breakpoint chlorination (WR 12) and final chlorination (WR 14) were consistently free of virus. The same conclusion is valid for treated and untreated conventional dam water samples (WD-series).

Enteroviruses comprised the single largest virus group. Of these, poliovirus was the commonest, having been isolated on 69 occasions as the only virus, but additionally from samples with mixed enteroviruses. All three types, namely 1, 2 and 3, of poliovirus were found. Tests to differentiate between 'wild' type and vaccine type poliovirus

were not done as the method available at the time and which involved the demonstration of subtle temperature sensitivity differences was technically difficult. It did not always yield consistent results, necessitating frequent repetition which was costly and time-consuming. Today the two varieties can be distinguished with much less expense and effort by the oligonucleotide and peptide mapping technique.

Figure 8 illustrates that virus isolation became progressively less common from settled sewage (WS 18) through humus tank effluent (WS 20) to maturation pond effluent (WR 1) and became altogether zero thereafter. Also shown are the zero negative viral results in conventional waters.

The reoviruses and the enterovirus group are both commonly responsible for enteric infections and were therefore selected as viral monitors in this project. Briefly they may be summarized as follows :

Reoviruses are double stranded segmented RNA viruses and the name is derived from the initials of 'respiratory, enteric and orphan'. These are ubiquitous viruses and are commonly isolated from healthy individuals, especially children. Most infections are believed to be asymptomatic but reoviruses may cause mild respiratory or gastrointestinal illness in children. The enterovirus group consists of single stranded RNA viruses and may be subclassified into poliovirus, coxsackievirus, echovirus and enterovirus.

Poliovirus is further classified into types 1, 2 and 3 of which type 1 is the most virulent and more often associated with paralytic poliomyelitis. The non-polio enteroviruses (coxsackie, echo and entero) as a group are also prevalent in the intestinal tract, especially in children.

Isolation rates vary from 5% in temperate climates to 50% in the tropics (Metselaar and Simpson, 1982). Some of the syndromes caused by this group are :

- 'Summer illness' characterized by fever and malaise of a few days' duration, sometimes a rash;

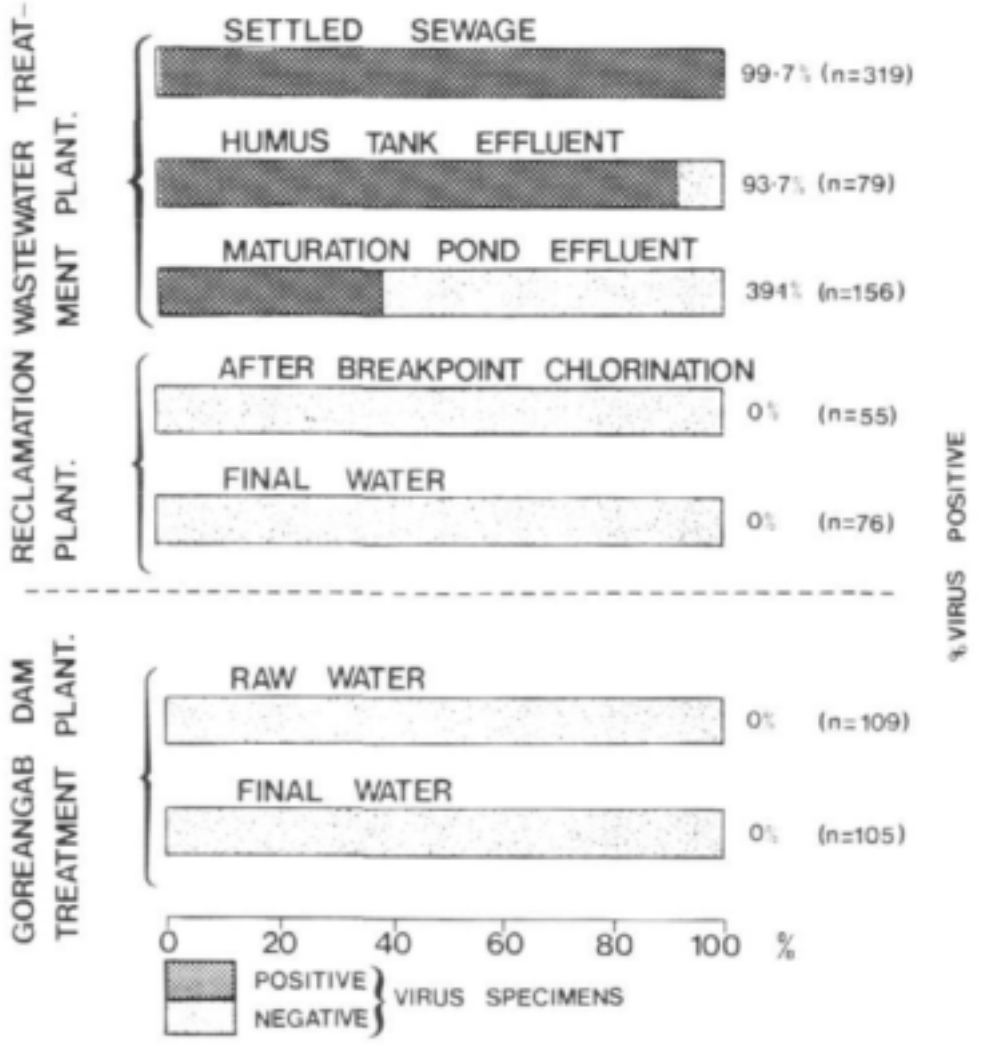

FIGURE 8: VIROLOGICAL ANALYSIS OF 685 SAMPLES TAKEN AT SUCCESSIVE STAGES OF WATER RECLAMATION AND 214 TREATED AND UNTREATED CONVENTIONAL WATER SAMPLES.

- aseptic meningitis, encephalitis and meningoencephalitis;

- respiratory infections;

- myocarditis, particularly by coxsackie B, was first recognized in newborns in whom it is frequently fatal and epidemics with high case fatality rates occur in maternity homes. This condition is now also recognized in older children and adults in whom it is less serious;

- epidemic myalgia, formerly known as 'pleurodynia' or 'Bornholm's disease', is associated with coxsackie B virus infection;

- juvenile-onset diabetes is thought to follow coxsackie B infection in some cases;

- herpangina, characterized by fever, sore throat, blisters in the mouth and vomiting is caused by coxsackie A virus;

- hand, foot and mouth disease is also due to coxsackie A virus;

- haemorrhagic conjunctivitis is caused by coxsackie A and by enteroviruses 68 and 71;

- gastroenteritis, although a causal relationship between these viruses and this syndrome has not been clearly established.

The overall virus isolation rate in this study was 50,4% i.e. 453 isolations from 899 samples tested. The viruses identified in the positive cultures are given in Table 3. Clearly, all of these viruses were not detected in conventional water sources prior to treatment but were highly prevalent in the source for reclaimed water as anticipated by the more intensive treatment process in the case of the latter.

Testing of reclaimed water has proven it to be consistently free of those waterborne viruses which were detectable by the most sensitive methods currently available (Grabow and Nupen, 1981; Grabow, Nupen and Bateman, 1984).

Table 3

VIRUSES IDENTIFIED IN 453 POSITIVE CULTURES DURING THE PERIOD JULY 1976 TO APRIL 1982

Virus identified	Number	%
Reovirus	106	23,4
Poliovirus	69	15,2
Coxsackie A virus	3	0,7
Coxsackie B virus	57	12,6
Echovirus	85	18,8
More than one enterovirus isolated	31	6,8
Unspecified enteroviruses	23	5,1
Reo and enteroviruses isolated	31	6,8
Unidentified viruses	48	10,6
Total	453	100,0

The failure to detect enteric viruses in the final reclaimed water indicates that this water is virologically safe. However, the methods used can only detect enteric viruses like reoviruses and enteroviruses such as polio, coxsackie B, echo and some coxsackie A viruses. The methods cannot detect the majority of coxsackie A viruses or the most important viruses in water hygiene, namely hepatitis A virus (HAV) and gastroenteritis viruses such as rota, norwalk and fastidious adenoviruses. Even though the absence of detectable viruses strongly suggests the absence of all viruses (IAWPRC Study Group on Water Virology, 1983), the shortcomings in virological technology emphasize the importance of epidemiological studies as a means of confirming that viruses are not transmitted by the reclaimed water.

6.2 Bacteriological studies

A large volume of analytical results was collected during this study and their inclusion would have rendered this report too bulky. Therefore the results for the entire period will be discussed by sampling point:

6.2.1 Waste water treatment

The main stages of waste water treatment are represented by sampling points WS 18 (settled sewage), WS 20 (humus tank effluent or secondary sedimentation) and WR 1 (maturation pond effluent and also raw water intake to reclamation plant).

As expected, the total plate counts (TPC, now known as standard plate counts SPC), total coliforms and faecal coliform counts were high. Faecal indicator organisms were present regularly. Table 4 summarizes the findings during the year 1 May 1981 to 30 April 1982 and is representative of these sampling points throughout the study. Vibrio cholerae was never isolated from any of the sewage samples.

6.2.2 Water reclamation

The improvement in the bacterial quality of the reclaimed water after the first two stages of reclamation (WR 1 and WR 3) is so vast that the quality of the other sampling points is not depicted in Table 4. They will be discussed separately.

- Primary settling (WR 3)
  The total counts, compared to those of WR 1 are greatly reduced. Faecal indicator organisms were present in low numbers and frequently absent.

- Secondary settling and recarbonation (WR 9 and 10)
  Total plate counts were generally very low but exceptions occurred. Coliforms were frequently absent or present in low numbers. Indicator organisms, especially E. coli, were usually absent.

- Breakpoint chlorination (WR 12)
  The number of samples tested at this stage in the reclamation process obviously varied greatly as a result of periodic plant closures. Not surprisingly, however, the various water quality tests usually yielded negative results or quantitatively low numbers of bacteria. This trend may be illustrated by the findings during the period May, 1981 to April, 1982 inclusive when 289 samples were tested. A total

Table 4

THE BACTERIAL QUALITY OF THE WATER AFTER DIFFERENT STAGES OF SEWAGE PURIFICATION AND INITIAL STAGES OF RECLAMATION DURING THE PERIOD 1 MAY 1981 TO 30 APRIL 1982

Determinand	Sampling point				
	WS 18	WS 20	WS 22	WR 1	WR 3
Total plate count/mℓ	$2{,}2 \times 10^6$ - $1\,350 \times 10^6$	1×10^5 - 353×10^5	9×10^3 - 124×10^4	152 - 134×10^4	0 - 12×10^5
Total coliforms/100 mℓ	20×10^6 - $4\,390 \times 10^6$	5×10^5 - 422×10^6	30×10^3 - 132×10^5	10 - 570×10^3	0 - 42×10^3*
Faecal coliforms/100 mℓ	$7{,}6 \times 10^6$ - 371×10^6	7×10^4 - 39×10^6	3×10^3 - 23×10^5	1 - 143×10^3	0 - 100**
Cl. perfringens/100 mℓ	14×10^3 - 560×10^3	7×10^3 - 250×10^3	$3{,}3 \times 10^3$ - 20×10^3	300 - 50×10^3	800***
Enterococci/100 mℓ	10×10^3 - $3{,}4 \times 10^6$	900 - 850×10^3	200 - 200×10^3	2 - 17×10^3	present in 24/111
No of samples analysed	138	138	85	107	111

WS 18 = Settled sewage
WS 20 = Humus tank effluent
WR 1 = Maturation pond effluent, also raw water intake to reclamation plant
WS 22 = Activated sludge effluent
WR 3 = After primary settling (reclamation plant)

* Usually < 10
** Usually < 3
*** One sample only

of 269 (93,1%) had a SPC of 100 CFU/mℓ with the majority (60,2%) yielding negative results. Coliforms were obtained only twice during the year, with counts of 1 CFU/mℓ and 2 CFU/mℓ respectively while faecal coliforms were seen only once and E. coli not at all.

- Activated carbon adsorption (WR 13)
  The general trend at this sampling point was an expected increase in bacterial counts, especially in the SPC.

- Final chlorination (WR 14)
  The final water showed bacteriological results comparable to, if not better than, those of treated water from conventional sources. During the period 1 May 1981 to 31 March 1983, the results on 354 samples may be summarized as follows :

  304 (86%) with SPC of < 100 CFU/mℓ
  33 (9%) with SPC of 100 - 1 000 CFU/mℓ
  17 (5%) with SPC of > 1 000 CFU/mℓ

Total coliform counts yielded positive results in 3 samples (129, 2 and 1/100 mℓ respectively). Faecal coliforms were present in one sample only and E. coli was consistently absent.

6.2.3 Surface water

- Raw Goreangab Dam water (WD 22)
  Standard plate counts ranged from 102 to 124 000 CFU/mℓ; mostly the values were moderately raised.

- Treated Goreangab Dam water (WD 23)
  The total plate counts were usually zero to 100 CFU/mℓ. Coliforms were occasionally present but faecal coliforms and E. coli only rarely.

6.2.4 Mixed treated final water (WR 14 and WU 23)

During the period 1 May 1982 to 31 March 1983 a total of 114 samples yielded SPC's as follows :

97 (85%) : < 100 CFU/mℓ
16 (14%) : 232 - 5 100 CFU/mℓ
1 (1%) : innumerable/mℓ

Pseudomonas aeruginosa was detected once but no other indicator organisms were present.

The bacteriological quality improved markedly along the processing line and also during the course of this study. The final water (WR 14) was of a quality comparable to the treated domestic water supply originating from a conventional source. The Windhoek reclaimed water is therefore considered safe for human consumption from a bacteriological point of view.

6.3 Diarrhoeal diseases

A total of 15 186 episodes of diarrhoeal disease (DD) was investigated during 1 August 1976 to 31 March 1983. The number of actual persons with DD was smaller as some of the patients (not calculated) had more than one episode of DD during the study period.

Bacteriological investigation of 15 186 faecal samples yielded bacterial (aerobic) intestinal pathogens on 2 972 or 19,6% of the samples. The two most common agents isolated were Salmonella and Shigella which together accounted for 81,8% of all isolates, in approximately equal numbers (Table 5). The remaining 80,4% would include parasites, viruses and organisms such as Campylobacter, Y. enterocolitica, and vibrios which were not tested for.

The commonest Salmonella species were S. typhimurium, S. isangi and S. enteritidis. Only one S. typhi isolate was obtained, not unexpectedly in view of its tendency to present with systemic signs and symptoms rather than diarrhoea.

Table 5

BACTERIAL PATHOGENS ISOLATED FROM 15 186 EPISODES OF DIARRHOEAL DISEASE DURING AUGUST 1976 TO MARCH 1983

Bacterium	No. of isolations	%
Salmonella	1 250	42,1
Shigella	1 179	39,7
E. coli	426	14,3
Salmonella/E. coli	35	1,2
Shigella/E. coli	34	1,1
Salmonella/Shigella	45	1,5
Salmonella/Shigella/ E. coli	3	0,1
Total	2 972	100,0

Of 13 499 stools received over the period August 1976 to May 1982 a total of 3 372 stools were analysed for their virological content. Less than 1% were positive. When the technology became available to detect rotavirus antigen by a simple EIA kit system, a rotavirus study was carried out during 1983; the results of this study are to be reported elsewhere but a few salient features are worth mentioning in relation to water reclamation.

A total of 585 stools from DD patients of all race groups and all ages were tested by means of a micro-ELISA (enzyme-linked immunosorbent assay) for the demonstration of rotavirus antigen. The overall positivity rate was 7%, with no significant difference in seasonal or racial/socio-economic incidence. Comparison of isolation rates by age, however, showed a significantly higher incidence in those aged 2 years and less than in the older age groups ($p < 0,05$ by Fisher's exact test). If the positivity rate in this study is extrapolated to the DD study as a whole, the 19,6% isolation rate of causative agents would increase to 26,6%.

Although this was a pilot study it appeared that rotavirus was a less common cause of DD in white patients from reclaimed water areas than in those from conventional water areas. The converse was obviously true for all causes other than rotavirus.

Figure 9 and Tables 6 and 7 show the diarrhoeal disease incidence rates by various parameters. The most significant of these is Figure 9 which compares the mean annual incidence rates of 13 009 childhood DD episodes over a 7-year period by nature of water supply and by major areas. Areas 23 (black) and 22 (coloured) show much higher rates than the other areas. Furthermore, there appears to be no obvious difference between the white reclaimed and white conventional water supply areas. This observation is firmly borne out in Table 6 which shows the annual DD incidence rates (all ages) per 1 000 population by water supply. In this table the denominator data for the conventional and reclaimed water supply areas were established annually. For example, during the year 1982, the denominators used for these two areas were 13 015 and 20 666 respectively. Two items of interest arise from this table. Firstly, the DD incidence rate progressively but steadily increased during the study period. We ascribe this to several factors which include :

- increase of total population from about 75 000 to about 100 000;

- emigration from Windhoek by members of the higher socio-economic groups (including civil servants) resulting in more thinly spread health and other services to a larger population;

- migration from rural areas into Windhoek of members of lower socio-economic groups.

All these factors contribute to a larger pool of infection, increased transmission potential due to crowding and decreased environmental sanitation.

The second observation is that, from a water supply point of view, the reclaimed product has consistently 'performed' as good as and frequently better than the conventional sources with regard to diarrhoeal

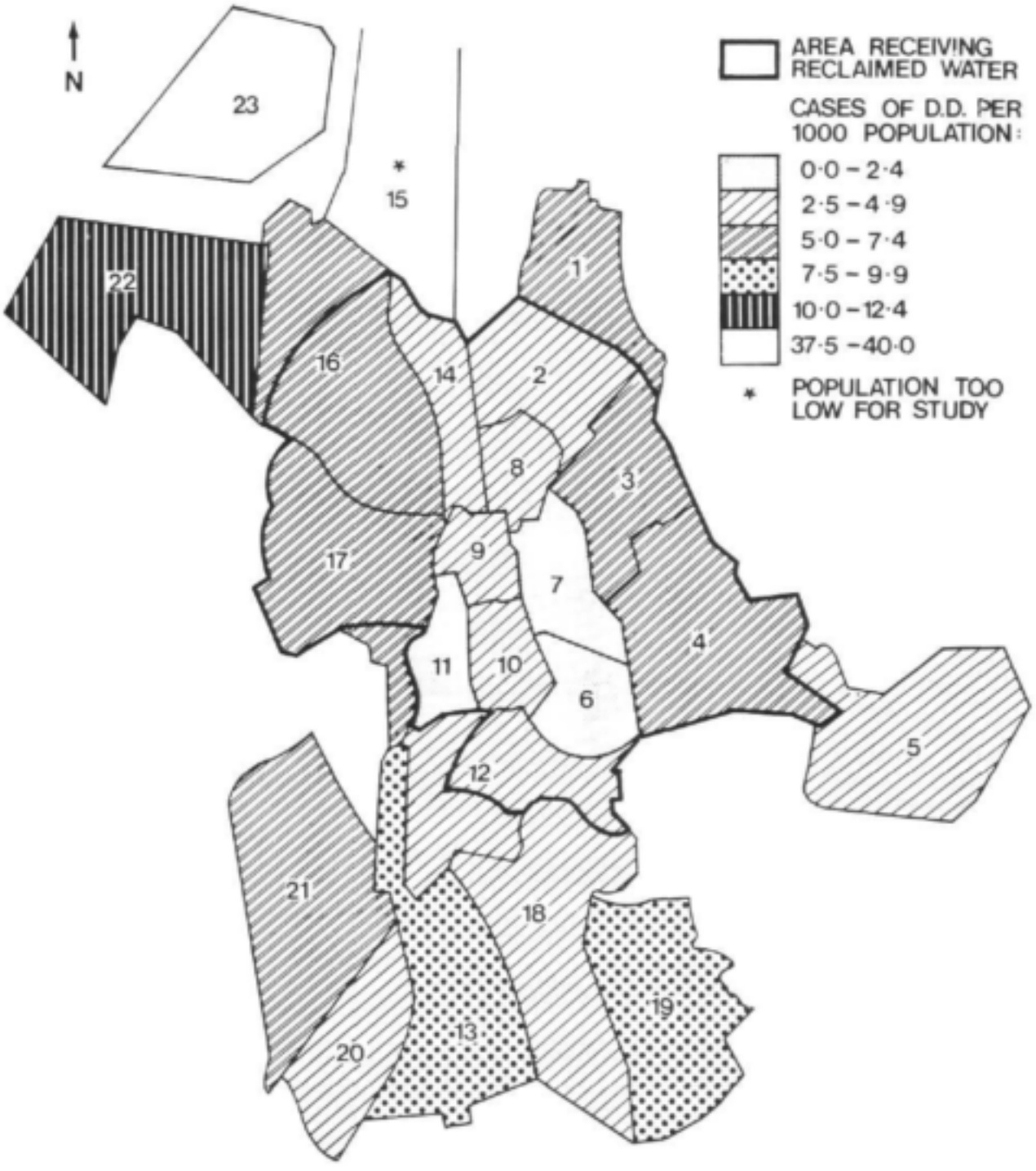

FIGURE 9: MEAN ANNUAL DIARRHOEAL DISEASE (DD) RATES IN CHILDREN OF 14 YEARS DURING 1 JANUARY 1977 TO 31 DECEMBER 1982 BY MAJOR AREAS.

Table 6

DIARRHOEAL DISEASE INCIDENCE RATES PER 1 000 WHITES (ALL AGES) BY CALENDAR YEAR AND BY WATER SUPPLY

Year	Nature of water supply		Difference
	Conventional	Reclaimed	
1977	6	4	Significant ($p = 0,03$)
1978	7	7	Not significant
1979	7	8	Not significant
1980	9	8	Not significant
1981	9	8	Not significant
1982	14	11	Significant ($0,001 < p < 0,01$)

Table 7

DIARRHOEAL DISEASE IN CHILDREN BY AGE AND ETHNIC GROUP - CUMULATIVE DATA (AUGUST 1976 TO MARCH 1983)

Age in years	White		Coloured		Black		Total	
	No. of cases	% *	No. of cases	% *	No. of cases	%	No. of cases	% *
< 1	364	31,7	904	50,2	5 356	53,2	6 624	50,9
1	207	49,7	465	76,0	2 737	80,4	3 409	77,1
2	138	61,8	166	85,2	871	89,1	1 175	86,2
3	83	69,0	59	88,5	378	92,9	520	90,2
4	50	73,3	39	90,7	190	94,8	279	92,3
5-9	196	90,4	105	96,5	386	98,6	687	97,6
10-14	110	100,0	63	100,0	142	00,0	315	100,0
	1 148		1 801		10 060		13 009	

* Cumulative

disease incidence.

On two occasions the difference was statistically significant. We conclude that the major differences shown between ethnic groups consuming the same water largely reflect differing socio-economic conditions as reflected by crowding, income, education and environmental sanitation (excluding the domestic water supply).

Table 7 presents supportive evidence in that it shows the classical age distribution of DD by ethnic group. Almost 90% of all childhood diarrhoeal disease in blacks occurred in children of 2 years and less. Comparable 90% levels were reached by white children between 5 and 9 years of age and by coloured children at 4 years. This phenomenon clearly reflects general socio-economic standards as referred to earlier.

It was recognized from the start that a number of persons living in conventional water supply areas commute to, and work in, reclaimed water areas since the latter includes the central business district. These are however a relatively minor proportion of the total population and furthermore adults in age groups which tend to have the lowest DD incidence rates. Children of schoolgoing age generally attend schools close to their home with a similar water supply. A major drawback in the Windhoek study, recognized from the start, is its low total population numbering 100 000. Of necessity this imposed the need to continue studies over a prolonged period of time in order to obtain the kind of data from which statistically significant conclusions could be drawn. (This particular drawback was felt more strongly in the mortality study). As far as the DD study was concerned, generally speaking, the white population is socio-economically reasonably homogeneous in that it consists mainly of civil service employees and the private business sector. This was clear from the 1975 census data. However, we attempted to find two areas which were as closely similar in all respects except water supply and selected major areas 03 (reclaimed water) and 19 (conventional water) and attempted to compare DD incidence rates in these two.

As a consequence of the 'small population factor' the incidence in in-

dividual suburbs such as these two was so low that statistically valid conclusions could not be drawn. Consequently we preferred using the population in toto despite the possible effects exerted by other variables.

The DDS aspect of the project was terminated during 1983, having served its purpose. We conclude that consumption of reclaimed water does not increase the risk of diarrhoeal diseases resulting from infectious agents. Indeed, there is a marginally lower incidence, an observation which is identical to that reported by the Los Angeles Health Effects Study (Nellor, Baird and Smyth, 1984).

6.4 Hepatitis studies

One of the major potentially waterborne viral infections is hepatitis A (HA). One of the objectives of the water reclamation health aspects study was to investigate patients with jaundice in Windhoek with a view to determining aetiology and possible association with water supply.

6.4.1. Phase I : 1976 to 1980

A total of 517 jaundiced patients were recorded. As there were relatively few coloured patients their data were combined with those of black patients. HBsAg tests were done on 327 patients and the results are shown in Table 8.

Table 8

THE RESULTS OF HBsAg TESTS ON 327 PATIENTS OUT OF 517 JAUNDICED PATIENTS DURING 1976 TO 1980

Ethnic group	HBsAg			
	Positive	Negative	Not tested	Total
Whites	5	115	58	178
Blacks/coloureds	60	147	132	339
Total	65	262	190	517

Approximately 20% of all patients tested were HBsAg positive indicating that the current illness was probably due to hepatitis B virus (HBV). However, there is a statistically significant difference between population groups in that few whites were HBsAg positive (4,2%) as compared with 29,0% among black and coloured patients. I.e. 96% of whites and 71% of blacks and coloureds were jaundiced as a result of causes other than HB. These could include hepatitis A virus (HAV). At this stage no further conclusions could be drawn.

6.4.2 Phase II : 1980 to 1982

Using the newer technology, more refined and specific results were obtained on a further 282 jaundiced patients out of 308 cases studied. The remaining 26 cases were either duplicated or there was insufficient material available for hepatitis A virus (HAV) tests.

Table 9 shows the HAV status by antibody type IgM (immunoglobulin M) and IgG (immunoglobulin G) and ethnic group of these 282 patients. Of these 282 patients 268 are further tabulated in Table 10 by age group. Table 11 lists the data of 36 patients with current HA infection (as shown by the presence of HA IgM antibodies) by date, type of water supply, ethnic group, age and sex. Table 12 shows the prevalence of HA IgG antibodies by age and ethnic group in 209 jaundiced patients. Table 13 records 23 patients (who have no evidence of either current or past HA) by date, type of water supply, ethnic group, age and sex.

These results show in the first instance that the hepatitis A status in Windhoek is very similar to that found in other Southern African populations. Of 135 jaundiced black patients of all ages, 123 (91%) had evidence of past HAV infection. The corresponding percentages for whites and coloureds were 52 and 87% respectively. Other studies in Southern Africa have shown up to 100% of (healthy, non-jaundiced) blacks to have IgG antibodies to HAV indicating past HAV infection and immunity (Prozesky, et al., 1980).

Table 9

HEPATITIS A VIRUS ANTIBODY STATUS BY ANTIBODY TYPE AND ETHNIC GROUP OF 282 JAUNDICED PATIENTS* STUDIED DURING 1981 TO 1982

Antibody status (interpretation)	Ethnic group								Total	%
	White	%	Coloured	%	Black	%	Not stated	%		
HA IgG present (past HAV infection)	45	52,3	41	87,2	123	91,1	11	78,6	220	78,0
HA IgM present (current HAV infection)	31	36,1	2	4,3	3	2,2	3	21,4	39	13,8
HA antibodies absent (susceptible to HAV infection)	10	11,6	4	8,5	9	6,7		-	23	8,2
Total	86		47		135		14		282	

*An additional 26 serum samples were either duplicates or insufficient for analysis.

Table 10

HEPATITIS A VIRUS ANTIBODY STATUS BY ANTIBODY TYPE, ETHNIC AND AGE GROUP OF 268 JAUNDICED PATIENTS

Age group	HAV antibody negative				HA IgM positive				HA IgG positive				Total		
	W		B/C		W		B/C		W		B/C		W	B/C	ALL
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	No.	No.
< 1			1	33							2	67		3	3
1			1	50			1	50						2	2
2			2	100	1	100							1	2	3
3							1	33			2	67		3	3
4			1	50							1	50		2	2
5-9			1	11	5	100	2	22			6	67	5	9	14
10-14	1	12			6	75			1	12	4	100	8	4	12
15-19	3	30			4	40			3	30	5	100	10	5	15
20-24	1	20			2	40			2	40	20	100	5	20	25
25-29	1	8	1	5	10	77			2	15	21	95	13	22	35
30-34					1	17			5	83	21	100	6	21	27
35-39			1	7	1	20			4	80	13	93	5	14	19
40-44	1	11			1	11			7	78	15	100	9	15	24
45-49			1	8					1	100	11	92	1	12	13
50-54									5	100	11	100	5	11	16
⩾ 55	3	17	4	11			1	3	15	83	31	86	18	36	54
Unknown											1	100		1	1
TOTAL	10	12	13	7	31	36*	5	2,7*	45	52**	164	90**	86	182	268

*Age adjusted rates for white(W) and black/coloured(B/C) are 30% and 2,5% respectively.
** Age adjusted rates for white and black/coloured are 56% and 91% respectively.

Table 11

DATA OF 36 PATIENTS* WITH ACUTE HEPATITIS A INFECTION (IgM POSITIVE) BY DATE, TYPE OF WATER SUPPLY, ETHNIC GROUP, AGE GROUP AND SEX DURING 1980 TO 1982

Month	Year	Major area code No. (C=conventional water R=reclaimed water)	Ethnic group (W=white C=coloured B=black)	Age group (in years)	Sex
Feb.	1980	23 C	C	5- 9	M
April	1980	16 R	W	10-14	F
April	1980	18 C	W	10-14	F
April	1980	21 C	W	25-29	M
Jan.	1981	10 R	W	25-29	F
July	1981	02 R	W	10-14	F
August	1981	23 C	C	3	M
August	1981	10 R	W	5- 9	F
August	1981	02 R	W	10-14	F
August	1981	21 C	W	25-29	F
Sept.	1981	23 C	B	5- 9	M
Sept.	1981	16 R	W	15-19	F
Oct.	1981	21 C	W	10-14	F
Oct.	1981	17 R	W	10-14	F
Oct.	1981	23 C	B	⩾ 55	F
Nov.	1981	17 R	W	5- 9	M
Nov.	1981	21 C	W	10-14	F
Nov.	1981	21 C	W	35-39	M
Dec.	1981	21 C	W	25-29	M
Jan.	1982	16 R	W	25-29	M
March	1982	23 C	B	1	M
March	1982	09 R	W	20-24	F
April	1982	17 R	W	25-29	M
May	1982	16 R	W	15-19	M
May	1982	17 R	W	25-29	F
May	1982	16 R	W	25-29	F
May	1982	10 R	W	25-29	F
May	1982	21 C	W	40-44	F
July	1982	16 R	W	5- 9	F
July	1982	21 C	W	5- 9	M
July	1982	18 C	W	15-19	M
August	1982	20 C	W	20-24	F
August	1982	21 C	W	25-29	M
Sept.	1982	08 R	W	30-34	M
Nov.	1982	21 C	W	2	M
Nov.	1982	21 C	W	5- 9	F

*No information was obtained on a further three HAV IgM positive jaundiced patients

Table 12

PREVALENCE BY AGE AND ETHNIC GROUP OF HEPATITIS A IgG ANTIBODIES (IgM NEGATIVE) IN 209 JAUNDICED PATIENTS DURING 1980 TO 1982

Age group in years	Ethnic Group			
	White	Coloured	Black	Total
< 1		1	1	2
1				
2				
3			2	2
4			1	1
5- 9		2	4	6
10-14	1	1	3	5
15-19	3	1	4	8
20-24	2	6	14	22
25-29	2	5	16	23
30-34	5	8	13	26
35-39	4	3	10	17
40-44	7	3	12	22
45-49	1	2	9	12
50-54	5	1	10	16
⩾ 55	15	7	24	46
Unknown		1		1
Total	45	41	123	209

Table 10 gives an indication as to the age at which the various ethnic groups become infected. In whites the peak incidence appears to occur between 5 and 29 years while in coloureds and blacks it tends to occur below the age of 10 years. This conclusion is based on IgM positive patients, i.e. those whose jaundice is due to current HAV infection. In view of the recent problems with age specific denominator data, the numbers of jaundiced patients were used as denominators. The age-adjusted overall prevalence rates of IgM (indicating current HAV infection) and IgG (past HAV infection and immunity) in whites on the one hand and blacks/coloureds on the other, did not differ significantly from the actually observed rates and may be summarized as follows:

	Whites	Blacks & Coloureds
IgM	29,8%	2,5%
IgG	56%	91%

Table 13

DATA OF 23 JAUNDICED, HA IgM AND IgG NEGATIVE PATIENTS, BY DATE, TYPE OF WATER SUPPLY, ETHNIC GROUP, AGE GROUP AND SEX, DURING 1980 TO 1982

Month	Year	Major area code No. (C=conventional water R=reclaimed water)	Ethnic group (W=white C=coloured B=black)	Age group (in years)	Sex
Feb.	1980	04 R	W	15-19	M
March	1980	09 R	W	⩾ 55	F
April	1980	22 C	C	5- 9	M
April	1980	20 C	W	40-44	M
April	1980	18 C	B	45-49	F
July	1981	22 C	C	⩾ 55	F
August	1981	20 C	W	25-29	M
Oct.	1981	09 R	W	20-24	M
Dec.	1981	23 C	B	2	M
Feb.	1982	17 R	W	⩾ 55	F
Feb.	1982	19 C	W	⩾ 55	F
March	1982	23 C	B	4	F
March	1982	Unknown	B	⩾ 55	F
March	1982	22 C	C	⩾ 55	F
April	1982	23 C	B	1	F
April	1982	23 C	B	1	F
April	1982	23 C	B	25-29	F
May	1982	22 C	C	35-39	M
June	1982	27 C	W	10-14	M
June	1982	04 R	W	15-19	M
July	1982	23 C	B	⩾ 55	F
August	1982	23 C	B	2	F
Sept.	1982	02 R	W	15-19	M

Our findings with regard to age of exposure to HAV are similar to those of other workers (Joubert et al., 1985 and Prozesky et al., 1980).

Table 12 shows much the same information but, in view of the relatively high occurrence of IgG antibodies, the three ethnic groups are presented separately and show that, not unexpectedly in view of their intermediate socio-economic niche, coloureds occupy a position between whites and blacks with regard to their 'prior experience' with HAV.

Tables 11 and 13 attempt to relate the incidence of HAV infection to water supply. This could be done to any extent only in whites, who are usually the only domestic recipients of reclaimed water.

Table 11 details the IgM positive jaundiced patients during the study period of more than 2 years. Only 39 such patients were identified and data were available on 36 of these. Analysis of these data yielded the following information: The number of white patients was 31 of whom 17 were on the intermittent reclaimed water reticulation and 14 received conventional water only. The white residential areas affected receiving conventional water were :

Area 18 (Suiderhof)	: 2 cases
Area 20 (Academia)	: 1 case
Area 21 (Pionier Park)	: 11 cases

A fairly high incidence of diarrhoeal disease in area 21 was noted but this was not further investigated at the time. Clearly, the HAV infections were geographically localized and were not related to water supply. Cases in reclaimed water areas were much more patchily distributed, 17 cases over 6 areas and in terms of numbers do not appear to be waterborne although the numbers are too low to permit dependable statistical significance testing.

Finally, patients who had never experienced HAV infection by virtue of their lack of both specific IgM and IgG antibodies were scrutinized (Table 13). These numbered 23 of whom 13 were black or coloured and 10 were white. Of the latter, 6 lived in reclaimed water areas and 4 in conventional water areas. In

view of the relatively long incubation period of hepatitis A, no attempt was made to relate onset of illness to periods of reticulation of reclaimed water.

We believe that our findings show that the consumption of reclaimed water did not result in the occurrence of hepatitis A and substantiate the results of Grabow et al. (1984).

During Phase II a more extensive study of hepatitis B was done since this was possible as a result of more refined techniques which gave more detailed and specific information. These included anti-HBs, anti-HBc and anti-HBe antibodies and HBs and HBe antigens by means of the bead solid phase RIA (Abbott, North Chicago) tests.

Table 14 classifies patients according to HBV as a possible cause of their current jaundice. In considering this aspect we have not included the various complications of HBV infection which may lead to chronic liver damage and eventually to jaundice as a late manifestation of such complications. This was felt to be entirely outside the scope of this report. Table 14 shows that hepatitis B starts manifesting itself at a much later age than hepatitis A. There are a number of young black children with evidence of current HB infection but very few with past HBV infection. The probable aetiology of jaundice in 282 Windhoek patients is summarized in Table 15.

Approximately half of all patients, irrespective of ethnic group, are jaundiced due to causes other than HAV or HBV. Such causes include other viruses, parasites, toxins (especially alcohol) and neoplasms as well as a variety of general medical and surgical conditions. Secondly, HAV is a rare cause of clinical jaundice in blacks and coloureds but much more common in whites. As elsewhere in Africa, most Windhoek blacks clearly acquire their HAV infection in asymptomatic or mild form in early childhood.

Table 14

HEPATITIS B (HB) STATUS OF 282* JAUNDICED PATIENTS AS DETERMINED BY HBV MARKERS, LIVER FUNCTION TESTS AND CLINICAL DATA DURING 1980-1982

Age	Current HB infection			Past HB infection			Current Non A-Non B**		
	W	C	B	W	C	B	W	C	B
< 1								1	1
1			2						
2			2	1					
3			2						1
4			2						
5-9	1	2	4		2			1	
10-14	4		3		1		1	1	1
15-19	3	1	2			2	3		3
20-24	3	4	14		2	2	1	2	9
25-29	3	2	10	4	2	8	2	3	9
30-34	2	3	4	2	4	9	3	5	5
35-39	1	1	5	1	1	3	3	2	8
40-44			4	3	1	5	7	2	7
45-49			3		2	6	1	2	7
50-54	1		1	1	1	7	4	1	18
≥ 55	5	3	9	5	6	17	13	7	2
Unknown		1	2						
Total	23	17	69	17	22	59	38	27	71

* 62 Patients are classified under 'Past HB' as well as non A - non B.

** For the purpose of this report 'non A non B' includes all causes other than HAV or HBV.

W = Whites; C = coloured; B = black

Table 15

THE PROBABLE AETIOLOGY OF 282 JAUNDICED PATIENTS IN THE PERIOD 1980 TO 1982

Ethnic group	Hepatitis A %	Hepatitis B %	All other causes %
Black	2,2	51,1	53,3
Coloured	4,3	36,2	57,4
White	36,1	26,7	44,2

Finally, evidence of past HBV infection was found in only 19,8% (17/86) of all white jaundiced patients but in as many as 46,8% (22/47) and 43,7% (59/135) of coloureds and blacks respectively. The much higher figures than during Phase I are largely attributable to the increased sensitivity of the tests.

Since only people ill with jaundice were studied, these figures do not reflect the HB status of the population as a whole. However, it was shown by Botha et al. (1984) that, during a general survey in northern SWA/Namibia an extremely high proportion of adults (84%) had evidence of past or previous HB, attributed to various factors including tribal practises, vector transmission etc. With such a large pool of viral HB our results in an urban setting in the same country are not surprising. Results similar to those of Botha et al. (1984) were obtained by Prosezky et al. (1983) in a rural Transvaal population in South Africa.

6.5 Mortality studies

It was considered desirable to include a study on long-term health effects, if any, imposed by the consumption of reclaimed water. There are indications that, especially in the field of cardiovascular and neoplastic disease, the hardness of the water and presence of various chemical compounds can be associated with variable prevalence rates of these diseases (Beresford, 1980; Kool et al., 1981; Kuzma, Kuzma and Buncher, 1977 and Page, Harris and Epstein, 1976).

No agreement exists on whether such associations are causal in nature and, generally speaking, no concensus of opinion has been reached on 'safe' levels of many compounds in water supplies. Drinking water is an emotive subject and many controversial opinions exist. The New Orleans drinking water controversy is representative of several such situations (de Rouen and Diem, 1975).

We recognized several pitfalls:

- the small size of the Windhoek population (ranging from approximately 75 000 to 100 000 during the study period) would yield low

numbers of deaths which, when classified by cause, would not be amenable to statistically valid analyses. It was concluded that, for any meaningful results to emerge, the study would have to be protracted over decades. This, in itself, would impose additional variables.

- for various reasons, the denominator data have become less useful and the population size and structure with major political changes ahead are likely to undergo sharp fluctuations.

The mortality study has been in progress for 7½ years (1 August 1976 to 31 December 1983) during which period exactly 3 000 Windhoek deaths have been analysed. Pre-natal deaths and deaths due to unnatural causes are not included in the study. In retrospect we believe this to be an error. Table 16 lists the more common causes of death (in

Table 16

SELECTED CAUSE-SPECIFIC PROPORTIONAL MORTALITY RATES BY ETHNIC GROUP DURING THE PERIOD 1 AUGUST 1976 TO 31 DECEMBER 1983 EXPRESSED AS PERCENTAGES OF THE TOTAL MORTALITY FOR EACH RACE*

Cause of death (WHO code)	Blacks % **	Coloureds % ***	Whites % ****
Diarrhoeal disease (B3)	19,1	18,8	1,4
Respiratory tuberculosis (B5)	9,6	5,3	0,5
Measles (B14)	1,3	0,6	0,2
Diabetes mellitis (B21)	0,7	1,2	3,4
Nutritional (B22)	2,7	2,1	
Meningitis (B24)	3,1	1,5	0,2
Hypertensive disease (B27)	1,5	2,1	1,2
Ischaemic heart disease (B28)	0,2	2,3	14,6
Congestive cardiac failure (B29:427)	7,5	8,5	12,3
Cerebrovascular disease (B30)	5,2	8,2	12,2
Bronchitis, emphysema, asthma (B33)	0,7	1,8	3,3
Ill-defined conditions (B45)	9,1	7,6	11,6
All malignant neoplasms (B19)	12,2	12,3	20,7
All other causes	27,1	27,7	18,4
Total	100	100	100

* Unnatural and pre-natal deaths have been excluded from the total mortality.
** Total Black deaths : 2 075
*** Total Coloured deaths : 341
**** Total White deaths : 584

Windhoek) expressed as a proportional rate of the total mortality by race. These rates have the obvious disadvantage of mutually influencing each other but were in our view nevertheless preferable to the use of total population denominators. The major causes of death, classified according to the WHO International Lists of Diseases (1948), in the three main ethnic groups are summarized in Table 17.

Table 17

THE MAJOR CAUSES OF 3 000 DEATHS IN THE WINDHOEK POPULATION OVER A PERIOD OF 7,5 YEARS (1 AUGUST 1976 TO 31 DECEMBER 1983)

Cause of death (1948 WHO code where applicable)	Black* %	Coloured** %	White*** %
Cardiovascular diseases	14,5	21,1	40,1
Malignant neoplasms (B19)	12,2	12,3	20,7
Diarrhoeal diseases (B3)	19,1	18,8	1,4
Respiratory tuberculosis (B5)	9,6	5,3	0,5
All other causes	44,6	42,5	37,3
Total	100	100	100

* Total Black deaths : 2 075
** Total Coloured deaths : 341
*** Total White deaths : 584

These findings are characteristic in that the leading causes of death in the socio-economically more advanced whites were cardiovascular disease and cancer, together accounting for more than 60% of all white deaths.

In blacks and coloureds, diarrhoeal diseases were a major cause of death.

Table 18 considers cancer mortality by site and by ethnic group. For these organ specific cancer mortality rates, the total deaths due to cancer were used as denominator data. The trend observed earlier in the study was maintained. Carcinoma of the lung is the commonest of all neoplasms in all races, only slightly exceeded in blacks by carcinoma of the oesophagus.

According to other workers quoted by C Isaacson (1982), lung cancer is one of the commonest tumours in black males in Natal, comprising up to 20% of all male neoplasms.

Table 18

SELECTED CANCER MORTALITY RATES BY SITE AND ETHNIC GROUP EXPRESSED AS A PERCENTAGE OF TOTAL CANCER DEATHS FOR EACH ETHNIC GROUP FOR THE PERIOD 1 AUGUST 1976 TO 31 DECEMBER 1983

Site (1948 WHO code)	Blacks* %	Coloureds** %	Whites*** %
Tongue (141)	2,4	2,4	-
Oesophagus (150)	14,6	7,1	0,8
Stomach (151)	6,7	4,8	9,1
Large gut excl. rectum (153)	2,0	-	3,3
Rectum and rectosigmoid (154)	2,0	-	3,3
Primary liver (155)	9,8	9,5	9,1
Trachea, bronchus, lung (162)	14,2	19,0	18,2
Breast (174)	3,9	14,3	3,3
Cervix (180)	9,1	7,1	1,7
Uterus (182)	0,4	2,4	-
Prostate (185)	3,5	4,8	4,1
Bladder (188)	0,8	2,4	2,5
Hodgkin's disease (201)	0,4	4,8	-
Leukaemia (204-207)	2,0	4,8	3,3
Other	28,2	16,6	41,3
Total	100	100	100

* Total Black deaths : 254
** Total Coloured deaths : 42
*** Total White deaths : 121

Carcinoma of the cervix uteri was the commonest neoplasm in black females in this study, whereas carcinoma of the body of the uterus was virtually non-existent in this population group. These findings, too, are consistent with those of other authors reporting on ethnic differences in cancer prevalence.

Similarly, malignancy of stomach, large bowel and rectum appears to be more common in Windhoek whites than in blacks. Breast cancer, which elsewhere is far more prevalent in white than in black women, was only slightly more common in Windhoek white than in black women. It was however, very common in coloured women. It should be re-emphasized however that our numbers are too low to draw any firm conclusions other than that the cancer pattern in Windhoek appears to be similar to that seen in other studies.

It is essential therefore for these mortality studies to continue although this should be seen as part and parcel of the country's normal health statistics programme rather than purely in the context of the reclaimed water augmentation scheme (U.S. Department of Health, Education and Welfare, 1977).

When sudden departures occur from the expected prevalence trends, it is only this kind of ongoing study that may arouse the first suspicion that a harmful factor is operating in the environment.

7 OTHER RELATED STUDIES ON THE QUALITY OF RECLAIMED WATER

In addition to the epidemiological studies reported here and the surveillance maintained over the quality of the reclaimed water produced, a number of ancillary studies were carried out by the NIWR to assess the toxicological properties of the reclaimed water. For instance, the organic material adsorbed onto active carbon during adsorption was extracted with a solvent and subcutaneously injected into rats to test for carcinogenicity. The results were negative (Nupen and Hattingh, 1975). In a follow-up study, humus tank effluent, reclaimed water, tap and distilled water were fed to rats as their sole source of water. In addition spent and virgin active carbon were also mixed into their feed. The experiment was conducted for more than 30 months. Humus tank effluent was the only source causing deleterious effects in the animals (Van Rensburg et al., 1977).

The development of continuous automatic biological surveillance systems based on fish respiratory and behavioural responses to intoxication were reported by Morgan and Kühn (1974) and Morgan (1977;1979). Fish are more sensitive than man to a number of toxicants and therefore provide an early warning of the possible presence of toxicants.

The Ames Salmonella/mammalian microsome mutagenicity assay (Ames, 1979) is now well established to detect mutagens in water. This test has been applied to study the efficiency of the reclamation process to reduce mutagenic activity. No mutagens were detected in the final chlorinated reclaimed water (Denkhaus et al., 1979 and Denkhaus, Grabow and Prozesky, 1980). This observation contrasts with the pre-

sence of mutagenic activity in drinking water supplies after conventional treatment of river waters (Grabow, Denkhaus and van Rossum, 1980 and Grabow, Burger and Nupen, 1980). No evidence has been obtained that this observed mutagenic activity constitutes a health hazard.

Sensitive cell culture techniques were also used for the detection of toxicants and potential carcinogens in water (Kfir and Prozesky 1982 a,b,c). Although some transformation activity was detected in the final water, it was still of the same order as found in conventionally treated potable waters.

The occurrence of enteric viruses in the raw water source to a reclamation plant and their removal by the different treatment stages during reclamation have also been extensively studied (Grabow and Nupen, 1981 ; IAWPRC Study Group on Water Virology, 1983 ; Grabow et al., 1983; Grabow, Coubrough, Hilner and Bateman, 1984 and Grabow, Nupen and Bateman, 1984). The results of these studies have led to a proposal for the disinfection of water to prevent the transmission of microbial diseases (Grabow and Isaäcson, 1978).

The use of coliphages as indicators of the virological quality of polluted water has also been investigated (Grabow, Coubrough, Nupen and Bateman, 1984). It was found that the coliphage count did not always correlate with those of the enteric viruses, but that this test, in combination with the standard plate count and that of acid-fast bacteria (Grabow, et al., 1980) offered a practical and reliable indicator system for evaluating the virological safety of treated drinking water supplies - even if this water was directly reclaimed from waste water.

These studies provided support to the observations made during this study and in this way provide a comprehensive support to protect the health of the consumer.

A comprehensive list of publications on various aspects is outlined in the references (Section 8.2).

8 REFERENCES

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9 APPENDICES

APPENDIX 9.1

COUNCIL FOR SCIENTIFIC AND INDUSTRIAL RESEARCH

NATIONAL INSTITUTE FOR WATER RESEARCH

W26/5/4/1

GUIDELINES FOR THE MICROBIOLOGICAL QUALITY OF DRINKING WATER

by

W O K Grabow

for

Steering Committee for Health Aspects of Water Supply:
Subcommittee for Preliminary Water Quality Criteria,
Microbiology Working Group

PRETORIA
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1. INTRODUCTION

The international literature contains an overwhelming and confusing variety of views, interpretations, definitions, terminology, techniques and recommendations for the microbiological quality of drinking water. This literature has been carefully evaluated, and water quality authorities locally and abroad have been consulted on their views and experience in order to establish guidelines for drinking water of acceptable microbiological quality as well as practical test methods.

This report contains guidelines for the microbiological quality of drinking water, notes on various aspects related to drinking water, definitions, recommended methods and selected references to associated literature.

2. RECOMMENDED LIMITS FOR THE MICROBIOLOGICAL QUALITY OF DRINKING-WATER

Guidelines for selected micro-organisms are listed in Table 1. It is not necessary to include all the tests in routine analysis of many drinking-water supplies. Tests for total coliform bacteria and the standard plate count should always be done since they are practical and sensitive indicators of unforeseen treatment failure or pollution. The other determinands may yield valuable additional evidence in certain cases such as untreated groundwater or drinking water derived by conventional treatment of polluted water or the reclamation of wastewater.

Drinking-water supplies should conform to the guidelines in Table 1 when leaving the treatment plant. Water at the consumer's tap should be of similar quality. However, tapwater may display elevated standard plate and even total coliform counts which may be due to growth in the distribution system. Generally these organisms do not constitute a significant health risk. In case of doubt, or if secondary pollution of the distribution system is suspected, some of the other determinands should be included for better assessment of the quality of the water and the reason for elevated counts.

When total coliform bacteria are isolated from drinking water, they should be identified because the presence of Escherichia coli is almost conclusive evidence of faecal pollution while other coliforms may be of non-faecal origin.

Table 1

GUIDELINE LIMITS FOR THE MICROBIOLOGICAL QUALITY OF DRINKING WATER

Determinand	Recommended limit	Maximum permissible	Crisis limit
Standard plate count/1 mℓ	100*	1 000	10 000
Total coliform count/100 mℓ	0*	5	100
Faecal coliform count/100 mℓ	0*	1	10
Clostridium perfringens/100 mℓ	0*	1	10
Coliphages/100 mℓ	0*	10	100
Enteric viruses/10 ℓ	0	1	10

*In 95% of annual samples.

3. TESTING FREQUENCY

When a sample of drinking water exceeds the guideline values in Table 1, another sample from the same source should be analysed immediately, including tests for additional determinands if considered necessary.

Recommendations for the frequency of routine analysis of drinking-water supplies are still under investigation. Until further notice, the guidelines in Table 2 should be followed. During the rainy season, or when secondary pollution or treatment failure is suspected, testing should be carried out more frequently.

Table 2

RECOMMENDED FREQUENCY FOR THE MICROBIOLOGICAL TESTING OF DRINKING-WATER SUPPLIES

Population served	Minimum number of tests
More than 30 000	1 every day
100 000 to 300 000	20 every month
25 000 to 100 000	10 every month
10 000 to 25 000	3 every month
2 500 to 10 000	2 every month
Less than 2 500	1 every month

4. DISINFECTION OF DRINKING WATER

Guidelines for the disinfection of drinking-water supplies are limited to chlorine because it is the most commonly used water disinfectant in South Africa and sound information is available on its utilisation.

Drinking-water supplies can be considered of acceptable microbiological quality when exposed to a free chlorine residual of 1 mg/ℓ for 30 min at a pH of less than 8 and turbidity of less than 1 nephelometric turbidity unit. If the recommended pH and turbidity are exceeded, higher free chlorine residuals may be required.

5. SANITARY SURVEYS

Sanitary surveys are important and should not be neglected in the evaluation of the microbiological quality of drinking-water supplies. This would include inspections to locate potential sources of secondary pollution of water in distribution systems, unforeseen heavy pollution of raw water supplies, pit latrines near boreholes, and the efficient operation of water treatment systems. Care should be taken that treatment processes used are adequate for satisfactory treatment of the water concerned.

6. DEFINITIONS AND TEST METHODS

Methods other than those recommended may be used provided they have been proven to yield equivalent results.

6.1 Standard plate count

6.1.1 **Definition.** All those micro-organisms which produce a visible colony in a pour plate using a non-selective medium rich in nutrients and incubation at 35°C for 48 h. The count excludes many organisms such as obligate anaerobes and acid-fast bacteria which represent a significant proportion of viable bacteria in water.

6.1.2 **Purpose of test.** Gives an indication of the general microbiological quality of water. Particularly useful for evaluating the efficiency of disinfection processes in the treatment of drinking-water supplies as well as the detection of after-growth or secondary contamination in distribution systems.

6.1.3 **Test method.** Pour plate technique using petri dishes with a diameter of 85 to 90 mm, 1 mℓ of test sample or appropriate dilutions of test sample, 20 mℓ of good quality commercial yeast extract agar or nutrient agar, incubation at 35°C for 48 h, and counting all colonies.

6.2 Total coliform bacteria

6.2.1 **Definition.** All those bacteria which produce a colony with a typical golden-green metallic sheen within 20 to 24 h of incubation at 35°C on good quality commercial m-Endo LES agar. Bacteria which conform to this definition of total coliforms consist mainly of *Escherichia coli*, species of *Klebsiella*, *Citrobacter* and *Enterobacter*, and *Aeromonas hydrophila*.

6.2.2 **Purpose of test.** Gives an indication of the sanitary quality of water. All the bacteria concerned can be of faecal origin, but many of them can also multiply in certain water environments, while A. hydrophila is primarily associated with water. Whatever their origin, there are good reasons for not wanting to see them in drinking water. Coliforms recovered from drinking water should be identified because the presence of E. coli is almost conclusive evidence of faecal pollution. Membranes overgrown with non-coliform colonies are also evidence of undesirable drinking-water quality. Generally used for assessing the quality of drinking-water supplies.

6.2.3 **Test method.** Membrane filtration using good quality membranes with a diameter of 47 mm and suitable porosity (0,45 µm), Difco m-Endo LES agar or equivalent commercial growth medium, petri dishes with a diameter of 65 mm, and 100, 10 or 1 mℓ of test sample or 1 mℓ of appropriate dilutions of test sample, incubation at 35°C for 10 to 24 h without resuscitation procedures, and counting all colonies with a typical golden-green metallic sheen. Distinction of colonies is sometimes difficult, in which case doubtful colonies should be picked off and identified.

6.3 Faecal coliform bacteria

6.3.1 **Definition.** All those bacteria which produce a typical blue colony on M-FC agar within 20 to 24 h of incubation at 44,5°C. Bacteria which conform to this definition of faecal coliforms, consist mainly of E. coli, but may also include species of Klebsiella, Citrobacter and Enterobacter, and occasionally even other species. Some of these bacteria can multiply in certain water environments.

6.3.2 **Purpose of test.** Gives an indication of probable faecal pollution. More specific for faecal pollution than total coliforms which are a more sensitive indicator of the general sanitary quality. Generally used for assessing the level of faecal pollution in wastewater effluents and receiving rivers,

dams and marine environments, as well as raw waters used for the preparation of drinking water and the pre-selection of *E. coli*.

6.3.3 **Test method.** Membrane filtration as for total coliforms using Difco M-FC agar without rosolic acid, or equivalent commercial growth medium, incubation at 44,5°C for 20 to 24 h without pre-incubation or resuscitation procedures, and counting all blue colonies.

6.4 *Escherichia coli*

6.4.1 **Definition.** Coliform bacteria which yield a positive indole reaction at 44,5°C. Bacteria which conform to this definition generally consist exclusively of *E. coli* which is of almost definite faecal origin, but may also include species of *Klebsiella* which are not necessarily of faecal origin.

6.4.2 **Purpose of test.** Gives an almost conclusive indication of faecal pollution. More specific for faecal pollution than faecal coliforms.

6.4.3 **Test method.** Pick and purify coliform colonies from membranes in tests for faecal coliforms (or total coliforms) and perform indole test at 44,5°C for 24 h. Identity can be confirmed by means of API 20E or other suitable commercial test kits.

6.5 *Clostridium perfringens*

6.5.1 **Definition.** All those bacteria which produce a typical black colony on tryptose-sulphite-cycloserine agar when incubated anaerobically at 45°C for 20 h, are referred to as the group of presumptive *C. perfringens* organisms, which includes *C. perfringens*. The group of presumptive *C. perfringens* bacteria consists mainly of various species of the genus *Clostridium*.

6.5.2 **Purpose of test.** *Clostridium perfringens* is almost conclu-

sive evidence of faecal pollution. The other members of the group of presumptive C. perfringens can be of faecal origin, but they can also multiply in certain water environments. There are good reasons for not wanting to see any members of the group in drinking-water supplies. These organisms produce spores which are exceptionally resistant to unfavourable conditions. The presence of C. perfringens may therefore be a useful indicator of remote faecal pollution, while the absence of presumptive C. perfringens bacteria from treated drinking-water supplies is sound evidence of efficient treatment and disinfection.

6.5.3 **Test method.** Membrane filtration as for total coliforms using Oxoid TSC agar without egg yolk or equivalent growth medium. Membranes are placed face downwards on the growth medium and covered with a top layer of the same medium to improve anaerobic conditions. Plates are incubated in an anaerobic jar for 20 h at 45°C. Typical black colonies are counted and should be picked off for identification in order to determine the presence of C. perfringens.

6.6 Coliphages

6.6.1 **Definition.** All those bacteriophages which produce a visible plaque in a double-layer-agar plaque assay using E. coli strain C (ATCC 13706) as host and incubation at 35°C for 16 h. This definition includes a wide spectrum of coliphages, but excludes coliphages such as male-specific phages.

6.6.2 **Purpose of test.** Coliphages are valuable indicators of the incidence and behaviour of human enteric viruses in water because they generally outnumber enteric viruses in water, they generally are at least as resistant to unfavourable conditions and treatment processes, and they can be detected by simple, economical and rapid techniques. The absence of coliphages from treated drinking-water supplies is virtually conclusive evidence of the absence of enteric viruses.

6.6.3 **Test method.** Double-layer-agar plaque assay using petri dishes with a diameter of 85 to 90 mm, E. coli strain C (ATCC 13706) as host, incubation at 35°C for 16 h, and counting all plaques.

6.7 Enteric viruses

6.7.1 **Definition.** Viruses which multiply in the gastro-intestinal tract of warm-blooded animals. In water quality evaluation the definition is generally limited to those enteric viruses which produce a cytopathogenic effect in mammalian cell cultures. This includes polio, entero, echo, most coxsackie B and some coxsackie A viruses, but excludes viruses such as hepatitis, rota, norwalk and most coxsackie A viruses.

6.7.2 **Purpose of test.** Viral infections can be contracted from polluted water, and the behaviour of viruses in water environments and their response to water treatment processes may differ from that of some indicator systems used for assessment of the hygienic quality of water. Virus tests may therefore be useful for certain purposes such as evaluation of the efficiency of water treatment processes or indicator systems.

Since currently applied tests for enteric virus exclude the viruses of primary concern in water hygiene, their value is basically limited to that of a very expensive, time-consuming and cumbersome indicator function. Although these tests are of special importance in research, their application in the routine testing of drinking-water supplies is hardly justified.

6.7.3 **Test method.** Various methods for the recovery of viruses from water are acceptable. These include ultrafiltration methods and adsorption-elution procedures using media such as negatively or positively charged filters. Detection of recovered viruses is best done by means of tube dilution assays or inoculation of cultures in flasks using a sensitive cell culture system such as primary vervet monkey kidney cells or

the PLC/PRF/5 human hepatoma cell line. Inoculated cultures should be incubated for at least three weeks in order to ensure that slow-growing viruses are also detected.

7. RELATED LITERATURE

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FORM NO. [ ][ ][ ][ ][ ] (1 5)

APPENDIX 9.2

WATER RESEARCH EPIDEMIOLOGY DATA ON PATIENTS ADMITTED TO STUDY IN WINDHOEK S.W.A.

PATIENT'S HOSPITAL NUMBER ..... [ ][ ][ ][ ][ ][ ] (7 12)

MAJOR AREA [ ][ ] (14 15)

ENUMERATION AREA [ ][ ][ ] (16 18)

NAME OF PATIENT .....

HOME ADDRESS .....

PLACE OF WORK/SCHOOL .....

ETHNIC GROUP — 01 WHITE; 02 COLOURED; 03 BLACK [ ][ ] (20 21)

SEX — 1 MALE; 2 FEMALE [ ] (23)

AGE IN YEARS — 01 LESS THAN 1; 02 1; 03 2; 04 3; 05 4; 06 5 - 9; 07 10 - 14; 08 15-19; 09 20-24; 10 25-29; 11 30 - 34; 12 35 - 39; 13 40 - 44; 14 45 - 49; 15 50 -54; 16 55+ [ ][ ] (25 26)

HOSPITAL OR CLINIC [ ][ ] (28 29)

WARD [ ][ ] (30 31)

STATE	01	MEDICAL	01
T.B.	02	SURGICAL	02
MENTAL	03	OBST. & GYN	03
KHOMASDAL CLINIC	04	INFECT. DISEASE	04
KATUTURA CLINIC	05	PAEDIATRIC	05
PRIVATE CATHOLIC	06	T.B.	06
ELIZABETH HOUSE	07	PSYCHIATRIC	07
PRIVATE CONSULTING ROOM	08	O.P.D./CASUALTY	08
		OTHER	09

DATE OF ADMISSION ..... [ ][ ][ ][ ][ ][ ] (33 38)

DIAGNOSES ON ADMISSION ..... [ ][ ] (40 41)

DATE SPECIMEN COLLECTED ..... [ ][ ][ ][ ][ ][ ] (43 48)

TYPE OF SPECIMEN FOR BACTERIOLOGY ..... [ ][ ] (50 51)

SENT FOR VIROLOGY [YES][NO] [ ][ ] (53 54)

LABORATORY RESULTS:

Culture yielded a) No pathogenic organisms ☐ [ ][ ] (55 56)

b) ..... [ ][ ] (57 58)

WATER RESEARCH EPIDEMIOLOGY

MORTALITY DATA

For official use only

FORM NUMBER [ ][ ][ ][ ][ ] (1–5)

NAME OF PATIENT .....HOSP. NO:..... [ ][ ][ ][ ][ ] (7–12)

HOME TOWN/DISTRICT .....

PLACE OF WORK/SCHOOL ..... MAJOR AREA [ ][ ] (14–15)

OCCUPATION .....

ETHNIC GROUP

01	02	03	04	05
WHITE	BASTER	COLOURED	HERERO	NAMA

06	07	08	09	10
DAMARA	OVAMBO	KAVANGO	HIMBO	CHIMBA

11	12
BUSHMAN	OTHER

..... [ ][ ][ ] (17–18)

SEX

1	2
MALE	FEMALE

..... [ ] (20)

AGE IN YEARS AT DEATH

06	02	03	04	05	06	07
LESS THAN 1	1	2	3	4	5 - 9	10 - 14

08	09	10	11	12
15 - 19	20 - 24	25 - 29	30 - 34	35 - 39

13	14	15	16
40 - 44	45 - 49	50 - 54	55 - and over

[ ][ ][ ] (22–23)

HOSPITAL

STATE	1
PRIVATE CATHOLIC	2
OTHER	3

[ ] (25)

DATE OF DEATH .....(Year/month/day) [ ][ ][ ][ ][ ][ ] (27–32)

CAUSE OF DEATH ..... [ ][ ] (34–36)

.....

.....

GENERAL CAUSES = 33

CERTIFIED BY DOCTOR .....CANCER = 22 [ ][ ] (38–39)

GASTRO/HEPATITIS STUDY COMPUTER CODES

Major areas		00-27
Enumeration areas		010-829
Ethnic group:	white	01
	black	03
	coloured	02
Sex	female	2
	male	1
	unspec.	0
Age in years	unspec.	00
	< 1	01
	1	02
	2	03
	3	04
	4	05
	5-9	06
	10-14	07
	15-19	08
	20-24	09
	25-29	10
	30-34	11
	35-39	12
	40-44	13
	45-49	14
	50-54	15
	> 55	16
Inpatients : ward nos.		01-07
Outpatients: - hosp.		08
- private		09
- other		10

Diag. on Adm.

- G.E./diarrhoea/etc.	28
- Inf. hep.	29
- jaundice/hep.fail./ cirrh. etc.	30
- CNS infection	31
- other & non-spec. diagnoses	32

Type of Specimen

Specimen:

CSF	33
Stool/rectal swab	34
blood	35

Lab. results:

Salmonella	36
Shigella	37
EEC	38
Meningococcus	39
Pneumococcus	40
H. influenza	41
T.B.	42
Aust. antigen	43
Coxsackie	44
Polio	45
No pathogen. bact.	46
Sent for virol.	47
Virol. Neg.	48
Virol. Pos.	49
Salm/E. coli	50
Shig./E. coli	51
Salm./Shig.	52
Salm./Shig./E. coli	53

APPENDIX 9.5

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for Medical Research

Hospital Street
PO Box 1038
JOHANNESBURG 2000

Telephone 725-0511 Telegrams BACTERIA

Die Suid-Afrikaanse Instituut
vir Mediese Navorsing

Hospitaalstraat
Posbus 1038
JOHANNESBURG 2000

Telefoon 725-0511 Telegramme BACTERIA

DATE OF COLLECTION / INSAMELINGSDATUM

TIME / TYD

FEE / GELDE

Sender / Afsender

Patient / Pasiënt

REF. NO. / VERWYSINGSNR

RECEIVED ON / ONTVANG OP

DATE OF REPORT / DATUM VAN VERSLAG

BIOCHEMISTRY REPORT / BIOCHEMIE VERSLAG

SERUM

CLASS / KLAS	TEST / TOETS	RESULT / RESULTAAT	REF. VALUES / VERW. WAARDES
2660	ALT		8 - 37 U/l
2750	AST		10 - 30 U/l
5060	HBD		193 - 343 U/l
2925	CK Total / Totaal		M 0 - 190 F/V 0 - 150 U/l
2935	CK MB		0 - 18 U/l
2941	CK ISOENZYMES / CK ISOENSIEME		
3130	LD		90 - 200 U/l
3260	ACP Total / Totaal		0 - 4.7 U/l
	ACP Tart. Lab.		0 - 1.6 U/l
2690	Adenosine Deaminase		U/l
3115	Aldolase		0.5 - 3.1 U/l
2720	Ammonia		M 20 - 57 F/V 14 - 46 µmol/l
3030	GLDH		M 0 - 4 F/V 0 - 3 U/l
3150	LAP		8 - 22 U/l
3180	Lipase		0 - 200 U/l
3190	Lysozyme		7 - 15 mg/l
	GPI		M 12 - 71 F 12 - 86 U/l

CSF/SSV

4200	Lactate / Melksuur		1.28 - 3.60 mmol/l
4170	Adenosine Deaminase		U/l

SERUM

CLASS / KLAS	TEST / TOETS	RESULT / RESULTAAT	REF. VALUES / VERW. WAARDES
2785	Bilirubin: Direct / Bilirubien: Direk		0 - 4 µmol/l
2790	Bilirubin: Total / Bilirubien: Totaal		4 - 21 µmol/l
5095	ALP		38 - 92 U/l
5040	γ GT		M 10 - 50 F/V 7 - 32 U/l
2660	ALT		8 - 37 U/l
2750	AST		10 - 30 U/l
5355	Total Protein / Totale Proteïene		W/B 55 - 80 B/S 62 - 83 g/l
2100	Albumin / Albumien		30 - 55 g/l
3145	SERUM ELECTROPHORESIS/ELEKTROFORESE VAN SERUM		

Albumin / Albumien	α_1	α_2	β	γ Globulins / Globuliene

The degree and direction of any abnormality is indicated by the arrows.
Die graad en rigting van enige afwykings word d.m.v. pyltjies aangedui.

RESULTS MAY NOT BE SATISFACTORY DUE TO / RESULTATE MAG ONBEVREDIGEND WEES WEENS

☐ Specimen too old / Monster te oud

☐ Insufficient specimen / Monster onvoldoende

☐ Haemolysis / Gehemoliseer

DATE/DATUM

TIME/TYD

TELEPHONED / GETELEFONEER

REPORT RECEIVED BY / VERSLAG ONTVANG DEUR

415

FOR DIRECTOR / NAMENS DIREKTEUR

BIOCHEM 10/80
P653017