

Health Aspects of Nitrate in Drinking-water and Possible Means of Denitrification (Literature Review)

J.W.H. ADAM

National Institute for Water Research, Council for Scientific and Industrial Research, P.O. Box 395, Pretoria, 0001

Abstract

Drinking-water containing nitrate in excess of 10 mg/l (as N) can cause a (sometimes fatal) blood disorder called methemoglobinaemia in infants under the age of six months, especially under three months. Nitrate *per se* is not toxic, but is the precursor to nitrite which is produced through microbial reduction of nitrate in the intestine or in food preparations, and which causes methemoglobinaemia. Children exposed to excessive nitrate in drinking-water can have slightly retarded bodily growth and slower reflexes. Cancer experts warn against prolonged use of high nitrate water by humans since it seems possible that under certain conditions, for example bladder infections, nitrate can be instrumental in the formation of carcinogenic nitrosamines within the human body. Vitamin C is considered to be an effective preventive agent or antidote against the health effects of nitrate or nitrite. Public water supplies should not, therefore, contain more than 10 mg/l nitrate (as N).

Of all the known treatment processes, ion exchange seems to be the most effective and economical for the removal of nitrate from drinking-water in South and South West Africa, where in the case of the latter excessive concentrations of nitrate often occur in groundwater. Solar distillation appears to be suitable for small-scale production of potable water.

Introduction

Groundwaters in certain parts of South Africa and South West Africa (SWA) contain high levels of nitrate and are considered to be unsuitable for human consumption for this reason alone.

Infants are susceptible to nitrate poisoning, which can be fatal if untreated. Since 1945, approximately 2 000 cases of infant methemoglobinaemia associated with high nitrate concentrations in drinking-water have been reported in world literature (ISCWQT, 1974). Although some infants were affected by water containing less than 10 mg/l nitrate (as N*), the majority were taken ill after drinking water containing more than 20 mg/l (Shuval and Gruener, 1972). About 10 per cent of these cases were fatal (ISCWQT, 1974). It is estimated that the cases reported represent only 10 per cent of those observed (Winton *et al.*, 1971). Despite this, these figures indicate that the disease is relatively rare.

Consumption of nitrate has no apparent short-term effects on adults. Tredoux (1975) noted that, in some parts of SWA, adults drink water with concentrations of 200 mg/l nitrate and higher, with no apparent ill effects.

This paper deals with the known effects of nitrate on human health, and the need and methods for denitrification of groundwater.

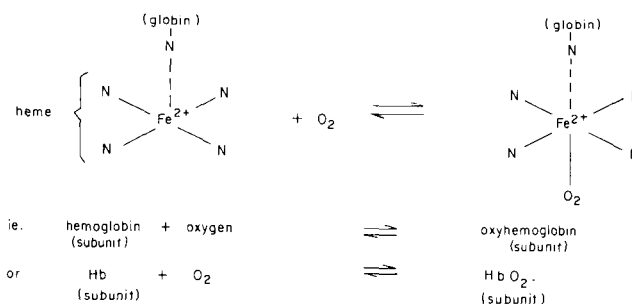
Effects of Nitrate in Drinking-water on Human Health

Methemoglobinaemia

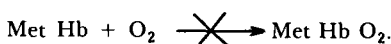
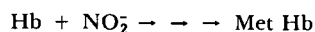
The consumption of inorganic nitrates and nitrites by infants, for example in the form of dried milk mixed with water containing nitrate, during the first six months of life (particularly the first three months) may cause methemoglobinaemia, resulting in cyanosis, which can lead to suffocation (ISCWQT, 1974).

Physiology

Methemoglobinaemia is a condition resulting from the conversion of hemoglobin (Hb), the oxygen carrier of mammalian blood, to methemoglobin (Met Hb), which is unable to transport oxygen (White *et al.*, 1968). The Hb molecule consists of four subunits, each of which is made up of a peptide chain (globin) and a heme group (with a ferrous, Fe²⁺, ion at the centre). Each subunit can reversibly bind and transport an oxygen molecule:



Several chemicals such as nitrites, perchlorates, sulphoamides and others (Shuval and Gruener, 1977) can cause conversion of Hb to Met Hb, in which the iron is in the ferric (Fe³⁺) state, rendering the molecule unable to bind oxygen. The exact mechanism of this conversion is still unknown. Schematically:



Nitrate, unlike nitrite, does not convert hemoglobin but can, under certain conditions, be reduced to nitrite by intestinal microflora, with subsequent formation of methemoglobin. Nitrate *per se* is non-toxic and is readily absorbed and excreted by the human body (Klotter, 1969; ISCWQT, 1974). Nitrate is thus only indirectly toxic in that it is the precursor to nitrite,

*All nitrate concentrations in this paper expressed as N.

which is poisonous owing to its property to form methemoglobin.

The finding that nitrate poisoning is limited to infants may be explained as follows (one or more of these explanations may apply in a particular case):

- (i) Conditions in the gastro-intestinal tract of infants are favourable for bacterial conversion of NO_3^- to NO_2^- . The capability to secrete gastric acid is incompletely developed in infants; consequently their stomach pH (4.6 to 6.5) is higher than that of adults (pH 2.0 to 5.0), permitting the presence of nitrate-reducing bacteria in the stomach and upper intestine. In addition, illness involving the gastro-intestinal system of infants, such as diarrhoea, may enable nitrate-reducing bacteria to move higher up the gastro-intestinal tract, increasing the likelihood of nitrite formation before the nitrate can be absorbed (Winton *et al.*, 1971; ISCWQT, 1974).
- (ii) Foetal Hb, which is still present in the new-born (60 to 80 % of the total Hb, decreasing to 20 to 30 % in three months) is more readily converted to Met Hb than adult Hb. Infants are also deficient in two enzymes in their red blood cells, namely Met Hb reductase and diaphorase, which convert Met Hb back to Hb (ISCWQT, 1974). Met Hb is normally present in human blood, but is kept at low, steady-state levels (1 to 2 % of the total hemoglobin) by the action of these enzymes. Clinical symptoms (cyanosis) become detectable at levels above 10 per cent, hypoxic signs and symptoms may develop at levels above 20 per cent, while death results at levels of 50 per cent and higher (ISCWQT, 1974).
- (iii) Babies consume about three times more fluid per unit body mass than adults (Winton *et al.*, 1971); this contributes to the hazard of nitrate-induced infant methemoglobinaemia, especially in arid climates where the fluid intake is higher than in moderate climates.
- (iv) Increased susceptibility to methemoglobinaemia may be due to hereditary defects in some individuals (White *et al.*, 1968).
- (v) Several cases of infant methemoglobinaemia have been traced to nitrate-rich vegetables such as spinach and carrots. Microbial reduction of nitrate to nitrite often takes place in infant food preparations prior to intake, especially when stored at room temperature (Klotter, 1969).

Maximum nitrate tolerance levels for infants

Since so many variables are involved in causing infant methemoglobinaemia, it is difficult to specify an infant tolerance level to nitrate in drinking-water. However, public health experts tend to agree that a limit of 10 mg/l provides a reasonable margin of safety (ISCWQT, 1974; *Water Quality Criteria 1972*; Shuval and Gruener, 1977; Winton *et al.*, 1971).

Effect of nitrate intake on children

There is evidence that children consuming high nitrate water tend to have increased Met Hb levels, this increase is inversely proportional to age. High Met Hb levels may be the cause of shorter than average heights (for the respective age levels (Takacs *et al.*, 1970), as well as slowing of conditioned reflexes in response to auditory and visual stimuli (Petukhov and

Ivanov, 1970). In the latter case the children under investigation, aged 12 to 14 years, had elevated Met Hb levels (5.3 % of total Hb) as a result of drinking water containing 24 mg/l NO_3^- . The control group drank water containing 2 mg/l NO_3^- and had only 0.75 per cent Met Hb (Petukhov and Ivanov, 1970).

Possible effects of nitrate intake on pregnant women

Studies with pregnant rats indicated that the passage of nitrite (not nitrate) through the placenta to the foetus resulted in raised Met Hb levels in the foetus. Puppies born to dams exposed to nitrite during gestation had impaired growth and development (Shuval and Gruener, 1977). These findings raise the question whether women should avoid drinking high nitrate water (with more than 10 mg/l NO_3^-) during pregnancy. No conclusive evidence concerning this matter is available.

Prevention and treatment of methemoglobinaemia

Since infants alone are directly affected by high nitrate water supplies, a practical precaution would be the use of bottled water from sources low in nitrate for the preparation of infant feeds. This practice was successfully applied in some small communities (Goodman, 1975; Klotter, 1969). However, Goodman (1975) warns of contamination which could pose an even greater hazard than nitrate to infants.

Ascorbic acid (vitamin C) is a very effective antidote to methemoglobinaemia as it reduces Met Hb in a direct, non-enzymatic reaction (Shuval and Gruener, 1977). Ascorbic acid also reduces nitrite to nitric oxide (NO), thus preventing the conversion of Hb by nitrite (Mirvish, 1977; Magee, 1977; Möhler, 1977). Infant food preparations containing vitamin C may reduce the incidence of the disease in areas where waters rich in nitrate are consumed (Shuval and Gruener, 1977).

Glutamate and glutathione exhibit remedial effects similar to those of vitamin C (Shuval and Gruener, 1977; Diskalenko *et al.*, 1977; Sen and Donaldson, 1974). Methylene blue has also been used quite successfully in the treatment of methemoglobinaemia; the action of this basic dye is attributed to the *in vivo* formation of an oxidative-reductive system (Sheehy and Way, 1974) which helps to reconvert methemoglobin to hemoglobin.

In conclusion, nitrate-rich drinking water (containing nitrate in excess of 10 mg/l) should be avoided, if possible. If this is impossible, vitamin C in the diet can be used as an effective antidote against the effects of nitrate on health.

Nitrate — A potential cancer hazard?

There is growing evidence that nitrate can play a part in the formation of carcinogenic N-nitroso compounds, in that it is a precursor to nitrite, which reacts with amines and amides to form N-nitroso compounds (Mirvish, 1977). This has, however, not yet been established conclusively, as can be seen from the following paragraphs.

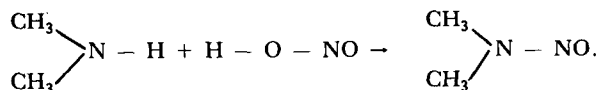
Carcinogenicity of N-nitroso compounds

N-nitroso (NNO) compounds (nitrosamines and nitrosamides) have possible carcinogenic, mutagenic and teratogenic properties (Shuval and Gruener, 1977). Cancer scientists have been using NNO-compounds for a number of years to induce tumours in various organs of many species of experimental animals (Mirvish, 1977). There is a definite relationship between the type of

substituents of NNO compounds, their carcinogenicity and their organ specificity (Möhler, 1977).

Chemical formation of NNO-compounds

In an acidic medium, nitrite reacts with secondary amines and N-substituted amides to form nitrosamines and nitrosamides, respectively, for example, dimethylamine reacts with nitrite (in the form of nitrous acid) to form dimethylnitrosamine:



The optimum pH for the nitrosation of secondary amines can be anything between 3 and 7, depending on the amine substituents (Möhler, 1977). Amides react more readily at pH values below 3 (Mirvish, 1977).

Nitrosation rates vary greatly. For example, N-substituted ureas and carbamates, secondary aromatic amines, weakly basic aliphatic secondary amines and tertiary enamines are more readily nitrosated than others (Mirvish, 1977).

Human gastric juice (pH 3,5) is an ideal medium for the nitrosation reaction (Lijinsky, 1976; Möhler, 1977). Experimental animals contract tumours when fed secondary amines and nitrite, but not when fed secondary amines and nitrate (Mirvish, 1977; Möhler, 1977). Since food contains both reactants, it is likely that nitrosamines are formed in food prior to or after ingestion (Mirvish, 1977). There is also evidence that nitrite is formed in human saliva by the action of oral bacteria (Möhler, 1977; Mirvish, 1977). Because the levels of nitrosamines detected in food have generally been low, endogenous formation of nitrosamines may present a greater potential cancer hazard than exposure to preformed nitrosamines (Lijinsky, 1976; Magee, 1977; Mirvish, 1977). The likelihood of nitrosamines persisting in lake and river water is small, since they are destroyed by ultraviolet radiation (Mirvish, 1977).

If the human bladder is infected (e.g. by *E. coli*), it can become a site for nitrosamine production and absorption, especially when the nitrate intake is high; for example when the drinking-water contains more than 20 mg/l NO₃ (Mirvish, 1977; Hill *et al.*, 1973).

As mentioned before, ascorbic acid reacts with nitrite and thus competitively inhibits (but does not eliminate) nitrosamine formation (Mirvish *et al.*, 1972; Möhler, 1977; Mirvish, 1977; Magee, 1977; Lijinsky, 1976).

Epidemiology

There is no epidemiological evidence that nitrosamines (or their precursors) are carcinogenic in man, but it is probable that man is sensitive to the carcinogenic action of these compounds (Magee, 1977). The type and quantity of nitrosamines which are carcinogenic in man have still to be determined, as has the amount taken in or produced in the body (Möhler, 1977; Magee, 1977). Epidemiological studies are complicated by the possible consumption by the subject of diverse carcinogens which induce the same type of cancer (Mirvish, 1977).

Correlations between the occurrence of gastric cancer and nitrate in drinking-water have been suggested on the basis of subjects who had been consuming water containing 21 to 33 mg/l NO₃ (Hill *et al.*, 1973; Hawksworth *et al.*, 1975). However Mirvish (1977) pointed out that these studies were strictly preliminary and should be extended.

In conclusion, the possible link between cancer and nitrate in drinking-water should caution against prolonged consumption of nitrate-rich water, but should not be cause for outright alarm at this stage.

Drinking-water Quality Criteria

Table 1 lists the maximum nitrate concentrations in drinking-water as recommended in several countries.

TABLE 1
RECOMMENDED NITRATE LIMITS IN SEVERAL
COUNTRIES (ADAPTED FROM HATTINGH, 1977;
PÖPPINGHAUS, 1975)

Country	mg/l**
USA (USPHS)* 1962	10
USA (NAS) 1972	10
USA (EPA) 1975	10
Japan 1968	10
SABS 1971	10
Australia 1973	10
WHO European 1970	<11,5
WHO International 1971	10
Britain	20
Russia	9,0
West Germany	11,3
Israel	10,2
Austria	9,0
East Germany	6,8
Denmark	5,7
Switzerland	4,5
Czechoslovakia	3,4

* USPHS = US Public Health Service
NAS = National Academy of Sciences
EPA = Environmental Protection Agency
SABS = South African Bureau of Standards
WHO = World Health Organization

**To convert the nitrate (as N) value to a nitrate (as NO₃) value, multiply by 4,43

Evidently 10 mg/l nitrate is regarded as a safe limit by the majority of health authorities (Winton *et al.*, 1971; ISCWQT, 1974; *Water Quality Criteria* 1972; Shual and Gruener, 1977). Mirvish (1977) noted that only when the concentration of nitrate in drinking-water reaches 20 mg/l will it become the main component of the total nitrate intake of adult persons, other sources of nitrate being, for example, vegetables and cured meat. This indicates that the limit of 10 mg/l NO₃ in drinking-water may be over-conservative for adults, but that it should not be raised to more than 20 mg/l NO₃.

It is therefore recommended that the maximum allowable nitrate-nitrogen concentration in public water supplies should not exceed 10 mg/l, and that adequate steps be taken to eliminate excessive nitrate concentrations in drinking-water. For small communities where better control over water usage is possible, it seems permissible to allow up to 20 mg/l nitrate in water used by adults and children, but sterile bottled water from sources low in nitrate should be used for infant food preparation. Breast feeding is still the safest and most natural way of raising infants. A varied diet, rich in vitamin C, is highly recommended

for the prevention of health complications caused by nitrate (or nitrite).

Occurrence of High Nitrate Groundwaters

The Department of Water Affairs has, in extensive surveys, identified certain areas in South Africa and South West Africa where groundwaters are rich in nitrate. Groundwater nitrate levels are rarely constant for long periods of time, and usually increase shortly after rains.

A thorough groundwater quality survey conducted by the National Institute for Water Research in SWA showed that 18 per cent of the total number of boreholes sampled contained nitrate in excess of 20 mg/l, the limit recommended in South West Africa for human consumption. This limit is rather high compared with the South African norm which allows 10 mg/l in drinking-water. Many more borehole waters would have been declared unfit for human consumption in South West Africa had the South African limit been applied. Data from the Department of Water Affairs indicate that, in the Springbok plains in the northern Transvaal, approximately 70 per cent of the borehole waters contain more than 10 mg/l nitrate.

The origin of nitrate in groundwater can usually be traced to contamination by percolating water carrying nitrate from sources such as —

- (i) decaying plant or animal material;
- (ii) agricultural fertilizers;
- (iii) domestic sewage;
- (iv) areas of high density animal confinement; or
- (v) geological formations containing soluble nitrogen compounds (Behnke, 1975; Nichols, 1965).

Water Treatment for the Reduction of Nitrate

These treatment methods can be classified broadly as physico-chemical or biological processes, some of which are used for general desalination, others being ion-specific.

Physico-Chemical Processes

Ion exchange

The nitrate-rich water is passed through a column containing ion exchange resin beads which can exchange nitrate ions with chloride ions present on the surface of the resin (Pöppinghaus, 1975). The resin must be regenerated regularly with a concentrated sodium chloride brine.

Advantages of ion exchange are that a high quality drinking-water is produced, that it is technically simple, and that the process has already been well developed.

Disadvantages are that it involves a fairly high capital outlay, requires regular attention and the isolation of an area for the disposal of regenerant brine. Because most ion exchange resins are not completely ion specific, the composition of some waters may be less suitable for ion exchange treatment owing to interference by other ions (e.g. sulphate; Macdonald and Potgieter, 1976).

After extensive ion exchange studies at the National Institute for Water Research (NIWR), only one commercially available resin was found suitable for the removal of nitrate from groundwater; however, evaluation of new resins is continuing*. Subsequent pilot plant tests at Aroab in South West Africa (Dalton, 1978), demonstrated that ion exchange is feasible for the production of drinking-water for small communities. A small household-scale ion exchange unit (similar to household water softeners currently on the market) costs between R50 and R100. Table salt (NaCl), used for regeneration (once or twice a month), is relatively inexpensive.

Reverse osmosis

This process, in simple terms, involves the application of external counterpressure, which is greater than the osmotic pressure, to squeeze fresh water out of a saline solution through a semi-permeable membrane. An 85 per cent removal of nitrate is attainable by reverse osmosis, but the process also removes most of the other salts present in the water. The feed water must be free of suspended solids or oils, which can clog the membranes (Ahlgren, 1976; Föppinghaus, 1975).

Unfortunately, reverse osmosis systems are very expensive, especially on a small scale, and while they can be justified if desalination of the product water is required, they cannot seriously be considered for nitrate removal.

Electrodialysis

In this process, ion-selective membranes are used in conjunction with an electric current to remove certain ions from water (Pöppinghaus, 1975). It is, however, not specific for nitrate and is generally used for desalination of brackish water. (Nitrate is removed together with the other soluble salts.)

Chemical reduction

Chemical reduction of nitrate to nitrogen gas can be achieved with various ferrous substances under specific conditions; for example, ferrous sulphate can act as reducing agent at alkaline pH values. About 50 per cent denitrification can be achieved by chemical reduction. The process has not been used extensively because of the high costs of chemicals and operation, and difficulties such as sludge handling and chemical inhibition (Macdonald and Potgieter, 1976; Pöppinghaus, 1975).

Distillation

Desalination by distillation has been applied in many technical process variations and refinements, but the cost of thermal energy makes large scale distillation uneconomical.

Owing to rising fuel costs, solar energy is becoming increasingly attractive. Solar distillation is used extensively in the USA and Australia, and should also be applicable in the hot South African climate. Since the yield per exposed surface unit area is rather low (about 1 to 6 l per m² per day) the capital outlay for solar stills is rather high and, at this stage, justifies only small-scale use (such as for households or small communities).

In collaboration with the NIWR, the Department of Water Affairs (1973) has developed a design for a solar still which is very easy to build and maintain (Van Steenderen, 1977). Extensive tests in South West Africa have shown it to be reliable

*Details available from the NIWR on request.

and weather-proof and to require very little supervision, apart from occasional flushing and cleaning (see Table 2 for technical details).

TABLE 2
TECHNICAL DATA OF SOLAR STILL*
(DEPARTMENT OF WATER AFFAIRS, 1973)

Situation on level ground:	
Length:	3 m
Width:	2,9 m
Height:	0,5 m
Evaporation area:	4,95 m ²
Approximate daily yield on hot southern African climate:	
Annual average	15 l/d
Minimum	6 l/d
Maximum	>27 l/d
Material costs (1978, Pretoria):	R120
*This solar still can be scaled up by increasing its length. The material costs will be increased by about R86 per additional length unit (3 m).	

Freezing

If water is partially frozen, relatively pure ice forms and the salts tend to stay in solution in the more concentrated brine, which remains unfrozen. The technical application of this concept is sophisticated, however, and lends itself to economical application only on a large scale.

Biological Methods

Algal ponds

Nitrate can be assimilated by algae. Cultivation of algae is a slow process, very much dependent on temperature and light intensity. Algal ponds must be shallow and exposed to light (Pöppinghaus, 1975).

Under optimal conditions 15 to 20 g algae can be produced per m² per day; 8,5 per cent of this will be nitrogen, which is assimilated as NO₃⁻, i.e. 1,3 to 1,7 g NO₃⁻ is assimilated per m² per day (De Wind, 1979). The separation of algae from water is difficult, and expensive techniques such as flotation, flocculation, centrifugation or microfiltration have to be used for this purpose (Pöppinghaus, 1975). It appears, at this stage, that algal ponds are not suitable for the production of denitrified potable water.

Bacterial denitrification

Some species of bacteria, commonly found in soil, are capable of using nitrate as a substitute for oxygen during respiration under anoxic conditions i.e. no available free dissolved oxygen (Focht and Chang, 1975). In this process, organic material serving as energy source is oxidized, while nitrate is reduced to harmless nitrogen gas.

Various forms of biological reactors can be used to bring denitrifying bacteria into contact with the water to be treated. These include attached growth reactors (e.g. packed anaerobic

columns, rotating disc units and fluidized bed columns) and suspended growth stirred tank reactors. High degrees of denitrification (over 90 %) are possible at retention times of about 30 min (or less) when optimal conditions are maintained; i.e. normal temperatures (15 to 30 °C), an adequate supply of carbonaceous material as energy source (such as methanol or cane sugar molasses), and good hydraulic conditions within reactors (Adam, 1979).

The main disadvantage of biological denitrification for the production of potable water is that the presence of biological sludge residues, colour and micro-organisms, make further treatment necessary to render the water suitable for human consumption. The water can, however, be used for stock watering.

Conclusions and Recommendations

According to the majority of recent publications on the health effects of nitrate, it seems prudent to recommend that public water supplies should not contain more than 10 mg/l nitrate. This limit strictly applies to infants (who can contract methemoglobinemia), but even children and adults should avoid prolonged consumption of water containing more than 20 mg/l nitrate. Therefore, it seems worth considering one or more of the above-mentioned methods for treatment of nitrate-rich drinking-water, either on a large scale to serve whole communities, or small scale to serve households. In either case ion exchange treatment appears the most feasible. Solar distillation should not, in spite of comparatively low yields of distilled water per unit area, be ruled out for small-scale use, since the stills are relatively easy to operate and maintain.

References

- ADAM, J.W.H. (1979) *Biological denitrification of groundwater*. National Institute for Water Research, Project Report No. 5, File No. W6/909/3.
- AHLGREN, R.M. (1976) Ultrafiltration, electro dialysis and reverse osmosis. *Wat. Sewage Wks*, Reference number, 1976, pp. R133-R138.
- BEHNKE, J. (1975) A summary of the biogeochemistry of nitrogen compounds in ground water. *J. Hydrol.* 27 155-167.
- DEPARTMENT OF WATER AFFAIRS (1973) *Handleiding vir die konstruksie van 'n dubbele skuins dak sondistillasie-eenheid*. Department of Water Affairs (SWA Branch), Windhoek.
- DALTON, G.L. (1978) *The removal by ion exchange of nitrates from borehole water at Aroab, SWA*. Internal project report, File no. W 6/1008/3 (Restricted), National Institute for Water Research, Pretoria.
- DE WIND, J. (1979) Personal communication.
- DISKALENKO, A.P., TROFIMENKO, Yu. N. and DOBRYAN-SKAYA, E.V. (1977) Prophylaxis of water-nitrate poisoning. *Zdravookhranenie* 20(3) 29-31. Seen in *Chem. Abs.* 87 166432 p.
- FOCHT, D.D. and CHANG, A.C. (1975) Nitrification and denitrification processes related to waste water treatment. *Adv. Appl. Microbiol.* 19 153-186.
- GOODMAN, A.H. (1975) Progress in methods of nitrate removal. *Wat. Treat. & Exam.* 24(3) 157-171.
- HATTINGH, W.H.J. (1977) Reclaimed water: A health hazard? *Water SA* 3(2) 104-112.
- HAWKSWORTH, G., HILL, M.J., GORDILLO, G. and CUELLO, C. (1975) Possible relationship between nitrates, nitrosamines and gastric cancer in SW Colombia. pp. 229-234 In: *N-nitroso compounds in the environment*, P. Bogovski et al. (eds.), Scientific Publication no. 9, International Agency for Research on Cancer, Lyon.

- HILL, M.J., HAWKSWORTH, G. and TATTERSALL, G. (1973) Bacteria, nitrosamines and cancer of the stomach. *Br. J. Cancer* 28 562-567.
- ISCWQT (1974) (International Standing Committee on Water Quality and Treatment). Nitrates in water supplies. *Aqua* 1 5-25.
- KLOTTER, H. (1969) Möglichkeiten zur Denitrifikation von Grundwässern. *Vom Wass.* 36 93-137.
- LIJINSKY, W. (1976) Health problems associated with nitrites and nitrosamines. *Ambio* 5(2) 67-72.
- MACDONALD, R.J. and POTGIETER, C.M. (1976) *Fundamental aspects of desalination*. Internal project report no. 1, File no. W 6/1007/3 (Restricted), National Institute for Water Research, Pretoria.
- MAGEE, P.N. (1977) Nitrogen as health hazard. *Ambio* 6(2-3) 123-125.
- MIRVISH, S.S. (1977) N-nitroso compounds, nitrite and nitrate: Possible implications for the causation of human cancer. *Prog. Water Technol.* 8(4/5) 195-207.
- MIRVISH, S.S., WALLCAVE, E., EAGEN, M. and SHUBIK, P. (1972) Ascorbate-nitrite reaction: Possible means of blocking the formation of carcinogenic N-nitroso compounds. *Science* N.Y. 177 p.65.
- MÖHLER, K. (1977) Krebs und Ernährung. *Umschau Wiss. Tech.* 77(8) 236-240.
- NICHOLS, M.S. (1965) Nitrates in the environment. *J. Am. Wat. Wks Ass.* 57 1319-1327.
- PETUKHOV, N.I., and IVANOV, A.V. (1970) Investigation of certain physico-physiological reactions in children suffering from methemoglobinaemia due to nitrates in water. *Hyg. Sanit.* 35 29.
- PÖPPINGHAUS, K. (1975) Chemisch-physikalische und biologische Massnahmen zur Stickstoffeliminierung aus dem Abwasser. *Gewässerschutz. Wasser. Abwasser* 17 77-105.
- SEN, N.P. and DONALDSON, B. (1974) The effect of ascorbic acid and glutathione on the formation of nitrosopiperazines from piperazine adipate and nitrite. pp. 103-106 In: *N-nitroso compounds in the environment*, P. Bogovski et al. (eds.), Scientific Publication No. 9, International Agency for Research on Cancer, Lyon.
- SHEEHY, M.H. and WAY, J.L. (1974) Nitrite intoxication: Protection with methylene blue and oxygen. *Toxicology and Applied Pharmacology* 30 221-226.
- SHUVAL, H.I. and GRUENER, N. (1972) Epidemiological and toxicological aspects of nitrates and nitrites in the environment. *Am. J. publ. Hlth* 62 1045-1052.
- SHUVAL, H.I. and GRUENER, N. (1977) Infant methemoglobinaemia and other health effects of nitrates in drinking water. *Prog. Water Technol.* 8(4/5) 183-193.
- TAKACS, S., VIGH, E. and GYURCSIK, A. (1970) Methaemoglobinaemie durch Trinkwasser und ihre Behandlung. *Städtehygiene* 5 114-117.
- TREDOUX, G. (1975) Personal communication.
- VAN STEENDEREN R.A. (1977) Studies on the efficiency of a solar distillation still for supplementing drinking water supplies in South West Africa. *Water SA* 3(1) 1-5.
- Water Quality Criteria 1972* (Report of the Committee on Water Quality Criteria, prepared for the EPA). Report no. EPA. R3. 73. 033, Washington, D.C.
- WHITE, A., HANDLER, P. and SMITH, E.L. (1968) *Principles of biochemistry*. Fourth edition, McGraw-Hill, Kogakusha.
- WINTON, E.F., TARDIFF, R.G., and McCABE, L.J. (1971) Nitrate in drinking water. *J. Am. Wat. Wks Ass.* 63(85) 95-98.