ENVIRONMENTAL HEALTH

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Health Hazards from Nitrates in Drinking–Water

HEALTH HAZARDS FROM NITRATES
IN DRINKING-WATER

ERRATUM

Please note that in sections 8.3.3 through 8.3.5, the
nitrate values referred to are expressed as NO_3 <u>not</u>
N as given in the note on terminology on page iv.

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HEALTH HAZARDS FROM NITRATES IN DRINKING-WATER

Report on a WHO meeting

Copenhagen
5-9 March 1984

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In 1981, the WHO Regional Office for Europe initiated a series of interim documents on chemical safety (Health Aspects of Chemical Safety), aimed at timely dissemination of information relating to diverse aspects of this rapidly expanding field. Between 2000-3000 copies of each of the 18 issues have been distributed worldwide, covering such topics as manpower development in toxicology, chemical accidents, legislation, pesticides, and combined exposures.

Based on the success of this endeavor, the WHO Regional Office for Europe has decided to broaden the scope of subjects covered. I am therefore pleased to announce the inauguration of the Environmental Health series, which will include topics related to water supply and sanitation, air and water pollution, radiation protection, food safety, occupational health, and chemical safety.

This first issue in the new series reviews the adverse effects of nitrates in drinking-water on public health which were discussed at a WHO working group held in Copenhagen in March 1984. Particularly due to intensification of agricultural production, with increasingly heavy application of nitrogenous fertilizers, the levels of nitrate in groundwater are considerably higher than they were a few decades ago. Compounding the problem is the long retention time in the unsaturated zones of aquifers. Thus, concentrations of nitrate in some sources of drinking-water are likely to continue growing for several decades, even if stringent controls are placed on future use of nitrogenous fertilizers.

The working group dispelled some of the exaggerated fears expressed about the potential adverse health effects, and its recommendations give pertinent guidance to Member States on the nature and extent of the problem and on an operational approach to its alleviation.

The World Health Organization recently published Guidelines for drinking-water quality, which, inter alia, contain recommendations concerning nitrates. The report of the WHO Regional Office for Europe Working Group on Health Hazards from Nitrates in Drinking-Water in no way changes or invalidates these recommendations but presents the experience and guidance of an eminent group of experts who are actively involved with this important aspect of drinking-water quality.

I hope that the new Environmental Health series will prove useful to those involved in both management and research.

J.I. Waddington
Director, Environmental Health
    Service

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THROUGHOUT THIS REPORT QUANTITATIVE REFERENCE TO NITRATE
IS EXPRESSED IN UNITS OF NITRATE-NITROGEN (NO_3-N)

CONTENTS

1. INTRODUCTION

A Working Group on Health Hazards from Nitrates in Drinking-Water was convened in Copenhagen from 5 to 9 March 1984 by the WHO Regional Office for Europe. It brought together 14 experts from 9 countries and a representative of the International Programme on Chemical Safety (Annex 2).

Mr J.I. Waddington, Director, Environmental Health Service, welcomed the participants on behalf of the Regional Director and briefly described current activities within the Regional Office's environmental health programme relating to drinking-water, particularly in connection with the International Drinking-Water Supply and Sanitation Decade. Dr R.F. Packham was elected Chairman, Dr O.M. Jensen Vice-Chairman and Professor S.A. Slorach Rapporteur.

Whereas in many regions of the world the main problem concerning drinking-water is to obtain adequate supplies, in Europe the main problems are due to chemical contamination. Increased application of artificial nitrogenous fertilizers, growing problems with animal waste disposal, changes in land use, and increasing concentration of nitrogen oxides in precipitation have resulted in marked increases in nitrate levels in drinking-water in some areas. Although WHO's drinking-water standards have been reviewed as recently as 1980, the situation with respect to nitrate needed to be reexamined.

The limits proposed or established by various organizations for nitrate in drinking-water are shown in Table 1. The current WHO drinking-water guideline value for nitrate is 10 mg/l. The WHO guidelines for drinking-water quality contain, inter alia, the following information on the nature of guideline values[1]:

"(a) When a guideline value is exceeded this should be a signal: (i) to investigate the cause, with a view to taking remedial action; (ii) to consult with authorities responsible for public health for advice.
(b) The guideline values specified have been derived to safeguard health on the basis of lifelong consumption. Short-term exposures to higher levels of chemical constituents, such as might occur following accidental contamination, may be tolerated but need to be assessed case by case, taking into account, for example, the acute toxicity of the substance involved.

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[1] Guidelines for drinking-water quality: Vol. 1. Recommendations. Geneva, World Health Organization, 1984, p.2.

(c) Short-term deviations above the guideline values do not
necessarily mean that the water is unsuitable for
consumption. The amount by which, and the period for
which, any guideline value can be exceeded without
affecting public health depend on the specific substance
involved.
It is recommended that, when a guideline value is
exceeded, the surveillance agency (usually the authority
responsible for public health) should be consulted for
advice on suitable action, taking into account the intake
of the substance from sources other than drinking-water
(for chemical constituents), the likelihood of adverse
effects, the practicability of remedial measures, and
other similar factors.

(d) In developing national drinking-water standards based on
these guidelines, it will be necessary to take account of
a variety of local, geographical, socioeconomic, dietary
and industrial conditions. This may lead to national
standards that differ appreciably from the guideline
values."

The aim of the meeting was to reappraise the health
significance of nitrate in drinking-water, taking into account
current trends in levels and data on the biological effects of
nitrate and related compounds.

To meet this aim, the Working Group undertook to:

-   examine the available information on nitrate levels in
    drinking-water supplies in Europe and determine trends;
-   assess the total daily intake of nitrates via food and
    water, and their bioavailability and metabolism;
-   examine the available evidence to determine if the presence
    of nitrates in drinking-water is associated with adverse
    health effects, including methaemoglobinaemia and gastric
    cancer;
-   review the technologies for lowering nitrate levels in
    drinking-water; and,
-   reappraise the present WHO guideline value for nitrates in
    drinking-water in light of the above.

Invited papers covering the above subjects were available
to the participants.

Table 1. Limits for nitrate in drinking-water

Organization	Year	Limit specification	mg/l of NO_3-N
WHO European standards	1970	Recommended	11.3
		Acceptable	11.3-22.6[a]
		Not recommended	> 22.6
WHO International standards	1971		10.2
WHO Working Group	1977	i. General population:	
		Acceptable	11.3
		Borderline	11.3-22.6
		Unacceptable	> 22.6
		ii. Infants < 6 months:	
		Unacceptable	11.3
US Environmental Protection Agency	1977	Maximum contaminant level	10.2
Health and Welfare Canada	1978	Maximum acceptable concentration	10.2
EEC (Directive on quality of surface waters intended for abstraction of drinking water)	1977	Imperative limit	11.3
		Guide level	5.6
EEC (Directive on quality of water for human consumption)	1980	Maximum admissible concentration	11.3
		Guide level	5.6
WHO guidelines for drinking water quality	1984	Guideline value	10

[a] Within this range, although a problem may not be apparent, physicians should be notified of the possible occurrence of infantile methaemoglobinaemia.

2. ANALYTICAL PROCEDURES AND DATA COMPARABILITY

2.1 Analytical procedures

Information collected from 12 European countries shows that most of them use standardized analytical methods to measure nitrate. These methods are usually based on photometric analysis after reduction. Table 2 shows a comparison of methods that can be used to determine total oxidized nitrogen and nitrate. Table 3 summarizes the analytical methods used by various countries.

New developments in ion selective electrodes may enable well-equipped laboratories to analyse nitrate and a number of other ions in relatively clear water samples easily and quickly.

2.2 Analytical quality assurance

In some countries, regular interlaboratory tests are carried out for analytical quality assurance. It is fundamental that analytical quality assurance, including interlaboratory calibration exercises, be carried out regularly to ensure the reliability and comparability of data.

2.3 Units used to express levels of nitrate, nitrite, etc.

Several different units are used to express the concentrations or amounts of nitrate and nitrite in water, biological fluids, foodstuffs, etc. For example, the quantity of nitrate may be reported as mg of NO_3, mg of NO_3-N, mmol of NO_3 or mg of $NaNO_3$. This difference in reporting has resulted in confusion, and great care is necessary if errors are to be avoided when comparing and collating the results of different studies. A convenient base for expressing nitrogenous compounds is nitrogen (e.g. NO_2-N, NO_3-N). To convert from NO_3-N to NO_3, the appropriate conversion factor is 4.429.

Table 2. Comparison of methods for determining total oxidized nitrogen and nitrate in water

	B	C	D	E	F	G
Method	Reduction/distillation (Devarda's alloy)	Continuous flow (copper/hydrazine)	Continuous flow (cadmium)	Visible spectrophotometry (sulfosalicylic acid)	UV spectrophotometry (direct measurement)	Ion-selective electrode
Type of sample	All, except saline	All, except saline	Saline	All, except saline	Nonsaline, low in organic matter	Raw and potable
Tested range[a] mg/l NO_3-N	12-40	0.02-36	0-0.175-0.56	0.2-10	0.1-9.0	1-50 (log-linear response)
Upper range[b] limit	10 mg in sample aliquot	40 mg/l without prior dilution	0.7 mg/l without prior dilution	5 ug in sample aliquot	80 ug in sample aliquot	1000 mg/l
Maximum concentration measurable using maximum sample aliquot	30 mg/l	40 mg/l	0.7 mg/l	0.2 mg/l	2 mg/l	1000 mg/l
Maximum sample aliquot ml	350	-	-	25	40	-
Limit of detection mg/l	0.2	0.01-0.26	0.0084	0.003-0.013	0.03	0.05-0.5
Time required for analysis[c] samples per hour	1	Up to 60	Up to 20	Up to 6	10	Up to 20
Comments	Can be performed after ammonia determination (on same sample portion by distillation). TOTAL OXIDIZED N METHOD	TOTAL OXIDIZED N METHOD	TOTAL OXIDIZED N METHOD. Low level range only.	METHOD FOR NITRATE ALONE	Interference from organic matter restricts sample applicability. Ideal for underground waters and as general sorting test. METHOD FOR NITRATE ONLY	Relatively poor precision. For use as sorting test for rapid monitoring. METHOD FOR NITRATE ONLY

[a] Tested range - range of concentrations for which standard deviation data has been obtained.
[b] Upper range limit - limit to which the method is know to give linear response. [c] Total analytical time.

Table 3.   Analytical methods used to determine nitrate in various countries

Country	Method	Reference
Belgium	Hydrazine sulfate method	See Table 2, method C
Bulgaria	Sulfuric acid and brucine Phenoldisulfuric acid	Bulgarian standard methods
Czechoslovakia		WHO recommended analytical methods
Denmark	a) Cadmium reduction b) UV method	a) See Table 2, method D b) See Danish standard methods 1975
Finland		Finnish standard methods according to standard methods for examination of water and wastewater. American Public Health Association (APHA), latest edition.
France		See APHA standard methods.
Germany, Federal Republic of	Sulfuric acid and brucine 2,6-Dimethexphenol	Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlamm-untersuchung: Verfahren DIN 38 405-Dq-1
Hungary	a) Sulfosalicylic acid b) Cadmium	a) See Table 2, method E b) See Table 2, method D (Hungarian standard methods)
Ireland		See APHA standard methods
Morocco	a) Sulfosalicylic acid b) cadmium	a) See Table 2, method E b) See Table 2, method D
Netherlands	Copper-cadmium column	See Table 2, method C Dutch standard methods
Norway	Cadmium reduction	See Table 2, method D
Sweden	Cadmium reduction Sulfuric acid and brucine	Swedish standard methods
Switzerland	a) Cadmium b) Ion-selection	a) See Table 2, method D b) See Table 2, method G
United Kingdom		See Table 2, methods B-F

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3. NITRATE LEVELS IN DRINKING-WATER AND TIME TRENDS IN EUROPE

3.1 Levels and time trends

Large-scale, long-term monitoring of drinking-water for nitrate has been carried out in only a few countries. Thus, most of the available data are fragmentary and, in many cases, do not permit statistically convincing conclusions to be drawn about trends in nitrate levels. Nevertheless, a number of general conclusions may be drawn about the situation in certain countries.

3.1.1 Belgium

During 1979-1982, the average concentration of nitrate in public water supplies was about 2.5 mg/l. Only 0.7% of the samples exceeded 10 mg/l, and in 10.7% of the samples, the level was between 5-10 mg/l. The study was limited to a number of waterworks in the northern part of Belgium, and the levels are considered representative for that whole area. Sampling has been carried out in southern Belgium, but those data have not yet been evaluated. However, average concentrations fluctuate between 0.2-3.9 mg/l. A regional study on private wells showed that 40% have nitrate concentrations above 11.3 mg/l. No indication of trends could be given. Total exposure cannot be calculated due to lack of data. The above figures extrapolated to the total Belgian population would result in exposure of about 456 000 persons to drinking-water with a nitrate level of 11.3 mg/l or above. (Data from D. Vethoeve).

3.1.2 Bulgaria

No recent data on nitrate levels are available. However, nitrate levels in groundwater sources are thought to have increased during the last few years. Exposure data for persons served by groundwater sources are as follows. In 1982, about 4% of the inhabitants in 243 settlements (375 594 persons) were exposed to water with nitrate levels above 11.3 mg/l and about 0.7% (68 settlements, 65 912 persons) were exposed to levels above 22.6 mg/l. Apparently, some increase in the number of inhabitants exposed to higher nitrate levels has taken place. At present, about 4% of the total population of 8.8 million is exposed to nitrate levels of 11.3 mg/l or above in drinking-water. (Data from P. Peneyatov).

3.1.3   Czechoslovakia

Countrywide data are not yet available. Based on present
estimates, about 1.8% of the total population of about 8 million
is exposed to nitrate levels above 11.3 mg/l. Most of the
drinking-water is derived from surface waters increasingly
polluted by nitrates from agricultural sources, particularly
fertilizers. The level is expected to increase by as much as
5% per year, but this increase may drop to 2% in the near
future. (Data from P. Grau).

3.1.4   Denmark

Data on nitrate concentrations were collected from
waterworks supplying about 99% of the total amount of
drinking-water. For the country as a whole, the water from 7.3%
of the waterworks exceeded the maximum admissible level for
nitrate (11.3 mg/l) either temporarily or permanently. A total
of 18% of all waterworks exceeded the Danish guideline level
of 5.6 mg/l. Large regional differences in concentrations
occur, with the western part showing significantly higher levels
than the eastern part.
        As population densities vary greatly throughout the
country, precise exposure data are difficult to assess.
However, about 373 760 persons are estimated to be exposed to
drinking-water with nitrate levels above 11.3 mg/l, and 921 600
to levels above 5.6 mg/l.
        Time-series analysis of nitrate concentrations in a
selected series of 184 groundwaterworks showed a rising trend of
0.2-1.3 mg/l/year (mean 0.75 mg/l). The rise started some
20-30 years ago and shows no sign of levelling off.
        (Data from J. Forslund and [1]).

3.1.5   Finland

A survey of Finnish waterworks in 1980 revealed that only
two water utilities (out of 699) produced drinking-water with a
nitrate level above 6.8 mg/l. No estimate of exposure could be
made due to lack of population data.
        Nitrate levels in water from private wells fairly often
exceed 6.8 mg/l, and in some cases, concentrations around
22.6 mg/l have been measured. Available data do not clearly
indicate whether or not the nitrate level has shown a tendency
to increase in recent years. (Data from K. Tapiolinna).

3.1.6 France

A survey on the nitrate problem in drinking-water was made during 1979-81 [2]. About 19% of the population is exposed to nitrate levels in drinking-water above 5.6 mg/l and 2-4% to levels above 11.3 mg/l. At most, 280 000 persons are exposed to levels above 22.6 mg/l at least once in 3 years. The highest nitrate levels are found in the northern and western parts of the country, particularly in groundwater in highly developed agricultural areas. About two thirds of the population is served by groundwater, and one third by surface water. The figures indicate a trend which could lead to the exposure of 2-5 million persons (4-10% of the population) to nitrate levels above 11.3 mg/l, the precise number depending on whether average or maximum values are taken into account.

3.1.7 Federal Republic of Germany

Around 28% of drinking-water is derived from surface waters which rarely contain nitrate levels exceeding 5.6 mg/l and usually show concentrations below 0.22 mg/l. However, these data are from 1978. According to some studies, the water from about 10% of all waterworks has a nitrate level above 11.3 mg/l; nearly all of these derive their drinking-water from groundwater. About 1% of the population, i.e. about 600 000 persons, receives drinking-water with a nitrate level that occasionally or permanently exceeds 20.3 mg/l. The available data do not directly indicate any trends but suggest that nitrate levels are increasing. (Data from H.W. Möller and [3,4]).

3.1.8 Hungary

At present, about 90% of the public water supply relies on groundwater, about half of it being bank-filtered surface water. About 7% of the total publicly supplied water contains nitrate around or above the Hungarian guideline level of 9.0 mg/l. Nitrate contamination is characteristic mainly for near-surface aquifers, especially in tertiary, quarternary and mesozoic layers with carbonate rock. Large problems exist in small settlements where private or individual wells are dug to the shallow aquifer. Around 316 000 people are served by 944 private wells, of which nearly 500 have very serious nitrate problems, 200 have frequent problems, and 200 have occasional problems with nitrate. A number of these private wells will be replaced by public supply. (Data from A. Homonnay).

3.1.9 Ireland

About 85% of the population of approximately 3 million is served by waterworks deriving water from surface-water sources. Small, private groundwater wells have only a few problems. Although nitrate levels have increased about twofold or less in Irish rivers, these levels do not pose a serious problem now or in the near future, as they are only 3-10% of the recommended limits. (Data from J. Daly and [5,6]).

3.1.10 Netherlands

About one third of the total population of approximately 14 million is served by waterworks using surface water. No nitrate problems are indicated in water from this source, the level being below 2.2 mg/l. Most of the remaining two thirds of the population is served by waterworks using groundwater. About 8.37 million people receive water containing nitrate levels less than 2.2 mg/l, 840 000 water containing 2.2-5.6 mg/l, and 56 000 water containing 5.6-11.3 mg/l.

Low concentrations of nitrate are found in water from some large waterworks which use surface waters containing rather high nitrate levels (Rhine water and Meuse water): the reduction in nitrate levels is due to denitrificaton during recharge in dune areas.

About 0.1% of the population is served by private wells, of which probably over 50% have nitrate levels exceeding 11.3 mg/l and an appreciable number have concentrations well over 22.6 mg/l and even as high as 33.9 mg/l.

At present, nitrate levels appear to be increasing in at least 20% of the wells, a trend expected to continue during the next 10-20 years [7]. However, not all wells will exceed the limit of 11.3 mg/l. For an indication of trends, see Reijnders et al. [8].

(Data from G.C.M. van Beck).

3.1.11 Norway

A survey of drinking-water quality has not yet been made. However, exposure to nitrate seems to be very low, as 85% of the population of about 3.5 million is supplied by waterworks based on surface waters having nitrate concentrations usually below 0.50 mg/l. The nitrate concentration in private wells sometimes exceeds the Norwegian guideline, but most groundwater has concentrations below 2.0 mg/l. Some increase is foreseen but not an alarming one. (Data from K. Ellingsen and [9]).

3.1.12 Sweden

Approximately half of the drinking-water is derived from surface water and half from groundwater. No problem exists with high nitrate levels in drinking-water derived from surface water. On the other hand, a recent report [10] estimates that at least 100 000 people use drinking-water from private wells with nitrate concentrations above 11.3 mg/l. In addition, public water supplies derived from groundwater in some areas in southern Sweden have levels above 11.3 mg/l.

During the last 20 years, nitrate levels in drinking-water derived from groundwater have increased in some agricultural areas in southern Sweden. However, insufficient data are available to determine how widespread this trend is. (Data from S.A. Slorach and [11]).

3.1.13 Switzerland

A recent study [12] indicates that about 98% of the population of 6.5 million is exposed to nitrate levels in drinking-water below 6.8 mg/l (average 2.3 mg/l). About 27% of the population is served by waterworks using surface waters, and these do not seem to pose a nitrate problem at present. On the other hand, nitrate levels in groundwater and well-water are increasing and have doubled since 1950 [13].

An inquiry among the communities revealed that the nitrate levels had increased in 18% of the waterworks, in 40% no data were available, and in no community did the nitrate level decrease. Nitrate problems are considered rare and limited to certain localities, and problems are not expected to increase in the near future. (Data from P. Michael, personal comm., and [14, 15]).

3.1.14 United Kingdom

Surveys have shown that a considerable number of raw water sources contain nitrate levels above 11.3 mg/l. Furthermore, increases in nitrate levels in surface water as well as in groundwater appear ongoing, leading to higher nitrate levels in drinking-water.

In total, about 7% of the population (i.e. 3.9 million persons) seems to be exposed to nitrate levels above 11.3 mg/l and about 36%, or 20.6 million, to levels above 5.6 mg/l. (Data from R.F. Packham and [16-22]).

3.2 Origin of nitrate

Nitrate in surface water and groundwater comes from several primary sources: the atmosphere, sewage and industrial effluents, agriculture, and natural sources. The following is a compilation and evaluation of the information collected from various European countries.

3.2.1 Atmosphere

The atmospheric contribution consists of nitrate and ammonia, and is usually considered to be the smallest. However, recent information on wet and dry deposition of pollutants, including nitrates and ammonia, suggests that the contribution may be considerable (up to 25% of the total load of nitrate) [23] and could well increase [24].

3.2.2 Sewage and industrial effluents

Some countries consider septic tanks, sewage and industrial effluents to be important contributors, but the effluents usually cause local (though sometimes quite serious) problems in groundwater and private wells and, through point source discharges, in surface waters. Percolation from waste disposal may also be considered as part of this category.

3.2.3 Agriculture

Agriculture is considered the main source of nitrates. All countries, even those which do not yet have any drinking-water problems, consider agricultural nitrate as their biggest problem. Nitrate is brought in large quantities onto the fields in manure, especially in stockbreeding areas, and fertilizers in intensive crop-producing areas.

3.2.4 Natural sources

Due to biodegradation of organic compounds in soil, naturally occurring nitrates are produced and released into the groundwater.
In general, nitrate reaches groundwater and surface water along two pathways. One pathway is run-off, especially from agricultural areas. The load coming from these areas varies with, for example, the season, and depends on such factors as temperature, slope, amount of rain and type of crop. The second pathway is infiltration. In agricultural areas, nitrate infiltrates into the soil, especially during autumn and winter

periods in bare fields and arable land with poor crop cover. In other areas, effluents from sewage and waste disposal (NH_4) also infiltrate into groundwater and thus may increase the nitrate load locally.

3.3 Fate of nitrate

The actual transport mechanisms and the various processes taking place before and during transport ultimately determine the fate of nitrate. The transport of nitrates depends, among others, on the following factors:

- actual amount of nitrate brought into an area, frequency of loading, and agricultural management;
- amount of water naturally or artificially deposited in the area;
- type of crop, crop rotation and agricultural practices;
- geological structure, climate, geochemical structure, permeability, and determining factors such as physicochemical characteristics, and soil conditions; and
- biological activity, i.e. denitrification of nitrate and nitrification of organic input.

The last-mentioned factor is of the utmost importance for the ultimate fate of nitrate. Under aerobic conditions, for example, in areas with large unsaturated zones, nitrate will be degraded or denitrified to a smaller extent but will percolate in large quantities into the aquifer. On the other hand, under anaerobic conditions, nitrate may be largely or completely degraded or denitrified.

Clearly, the presence of water, high or low water tables, amount of rain water, presence of other organic material giving rise to high biodegradation activity and presenting the necessary electron donor for denitrification, and some other physicochemical parameters are important conditions determining the ultimate fate of nitrate. In the Netherlands, Van Duijvenbooden & Loch [7] found that the risk of nitrate leaching into the deeper areas is much higher in sandy soils than in soils with finer textures (e.g. clay, peat). This increase is due to the higher permeability, higher aerobic activity and smaller amounts of organic material in sandy soil compared to soils such as clay.

In areas with sufficiently high water tables, withdrawal through a borehole may radically change a stable situation with respect to nitrate degradation in individual boreholes. By

lowering the water table in such a case, aerobic conditions may develop, leading to increased nitrification of the organic matter present in the soil.

3.4 Conclusions concerning nitrate levels and time trends

In most European countries, nitrate levels in drinking-water derived from surface waters seldom exceed 10 mg/1. However, problems have arisen in some areas, mainly due to run-off from agricultural land but also due to discharge of sewage effluents and certain industrial wastes. Nitrate concentrations in surface waters in many countries have increased substantially over the last 30-40 years, and especially in the last 20 years. Almost all countries studied showed a more marked increase in the levels of nitrates in groundwater, especially during the last decade. The main factors reponsible for this trend are increased use of artificial fetilizers, changes in land use, and disposal of waste from intensive animal farming.

Individual shallow wells in agricultural areas are particularly prone to contamination with nitrates, their levels frequently exceeding the WHO drinking-water guideline value of 10 mg/1. Poorly developed facilities for disposal of human and animal waste, which also give rise to poor hygienic water quality, appear to be the chief cause.

At present, several million people in Europe depend on drinking-water with a nitrate level exceeding 10 mg/1, and the number is likely to increase sharply in the next decade.

The rising trend in groundwater nitrate levels is likely to continue for several decades, even if nitrate leaching from soils is reduced by changes in agricultural practices.

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4. TOTAL DAILY INTAKE OF NITRATE

In general, diet is the major source of human intake of both nitrate and nitrite. Certain vegetables, e.g. lettuce, spinach, beetroot and celery, contain relatively high levels of nitrate (often over 226 mg/kg), but the nitrite levels are usually very low. Nitrates and nitrites are also added as preservatives in some foods, such as cured meats and certain types of cheese.

Daily dietary intakes of nitrate and nitrite have been estimated in different countries. [Ellen, G. & Schuller, P.L. Nitrate, origin of continuous anxiety. In: Preussmann, R., ed. Das Nitrosamin-Problem. Weinheim, Verlag Chemie, 1983]. The variation in the quantity of nitrates and nitrites ingested via the diet is extremely high. For example, individuals who seldom eat vegetables and cured meats have a low intake, whereas vegetarians have a relatively high intake.

In most European countries, the mean nitrate intake is about 10-30 mg/day. Vegetarians usually have a two- to four-fold higher intake of nitrates than nonvegetarians.

For most people in Europe, drinking-water does not contribute more than 30% of their total dietary intake of nitrate. However, when the nitrate level in drinking-water exceeds 10 mg/l, this contribution may be considerably higher.

For bottle-fed infants, drinking-water is usually the major source of dietary nitrate. Nitrate levels in breast milk are low, even when the lactating mother consumes nitrate-rich drinking-water. Breastfeeding should thus be encouraged, even in areas with high nitrate levels in drinking-water.

5. REDUCING DIETARY INTAKE OF NITRATE

In addition to lowering nitrate levels in drinking-water, other approaches can be used to reduce total dietary intake of nitrate. For example, various measures can be taken to reduce nitrate levels in vegetables. In the Netherlands, limits were introduced in 1982 for nitrate in lettuce, endive and spinach. The nitrate limit for lettuce grown in the open is 890 mg/kg, and the same limit applies to endive and spinach grown both under glass and in the open. Lettuce grown under glass may contain nitrate levels up to 1130 mg/kg. However, the Dutch Government intends to lower the nitrate limits for lettuce, endive and spinach to 565 mg/kg over a period of several years. A reduction of the very high levels of nitrate in these leafy vegetables should result in a marked decrease in dietary nitrate intake, especially in persons consuming large quantities of these foodstuffs.

Dietary intake of nitrate can also be reduced by advising the public to avoid regular consumption of large amounts of vegetables that routinely contain high concentrations of nitrate. However, such recommendations should be framed in such a way that they do not lead to a reduction in the overall consumption of vegetables. In Sweden, the food control authority has issued recommendations advising parents to avoid giving foods with a high nitrate content to infants less than 6 months old. These recommendations are intended to avoid the risk of methaemoglobinaemia that could arise if such infants are given, for example, spinach, beetroot or products thereof.
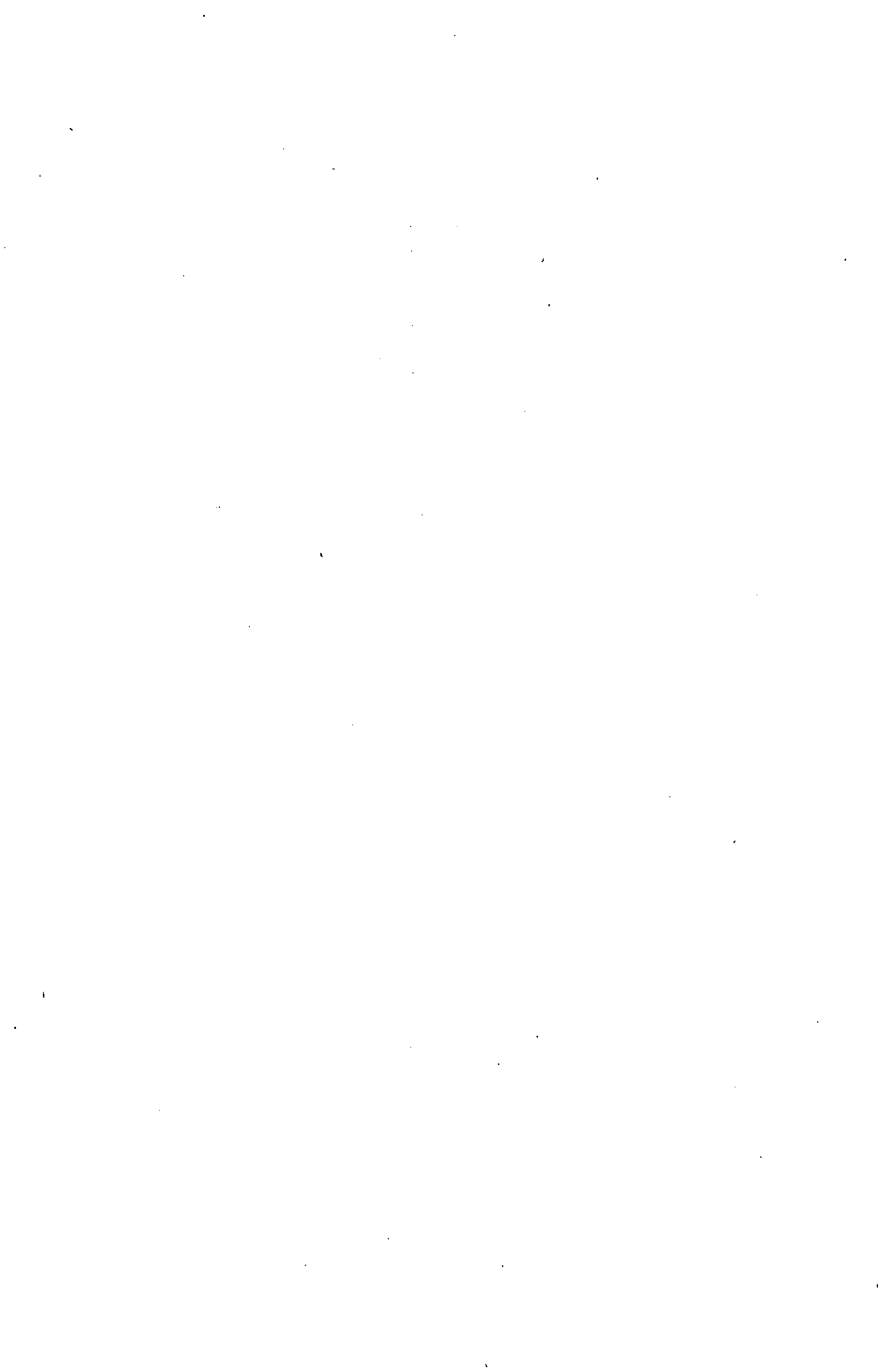
6. ENDOGENOUS FORMATION OF NITRATE, NITRITE AND N-NITROSO COMPOUNDS

6.1 Enzymology of nitrate reduction

The assimilatory reduction of nitrate to ammonia in plants and microorganisms proceeds in two separate, well-defined enzymatic steps:

(a) reduction of nitrate to nitrite, catalyzed by the flavomolybdoprotein, nitrate reductase;
(b) reduction of nitrite to ammonia, catalyzed by the iron-containing protein, nitrite reductase.

6.1.1 Nitrate reductase

Nitrate reductase, which catalyzes the reduction of nitrate to nitrite, has been isolated from bacteria, fungi, algae and plants. The enzyme contains molybdenum (1 atom or less/mol), and has a molecular weight of $1.6-6 \times 10^5$ daltons; the K_m for nitrate varies from 0.015-1 mmol/l [1]. In bacteria, one or both of two nitrate reductases, "A" and "B", are found [2]. Enzyme A reduces chlorate as well as nitrate and is more sensitive to cyanide and azide than enzyme B. Enzyme A is particulate, inducible by nitrate, and represented by O_2. Enzyme B does not reduce chlorate but is subject to chlorate inhibition and has a higher activation energy than enzyme A. Enzyme B is soluble and, when complexed, is unaffected by nitrate or oxygen. Most enzymes utilize nicotinamide-adenine dinucleotide phosphate (NADPH) or the reduced form of nicotinamide-adenine dinucleotide (NADH) as primary electron donors: enzymes from higher plants more often utilize NADH [3]. Methyl and benzyl viologens can serve (with few exceptions) as electron donors [2].

Nitrate reductase has the following enzyme functions:

- nitrate reduction using NADPH as an electron donor;
- nitrate reduction using methyl viologen, benzyl viologen, the reduced form of flavine mononucleotide ($FMNH_2$), or the reduced form of flavine adenine dinucleotide ($FADH_2$) as electron donors; and
- oxidation of NADPH using, e.g. cytochrome c, ferricyanide or tetrazolium as electron acceptors.

The separate functions have been found through isolation of nitrate reductase-defective mutants [4], and selective enzyme denaturation experiments [5]. Loss of molybdenum through

mutation or thermal denaturation, for example, leads to loss of ability to reduce nitrate, but cytochrome c reductase activity is retained [6]. Genetic evidence from <u>Aspergillus nidulans</u> [1] indicates that two 4.5 S flavin-bearing cytochrome c reductase subunits are united into a 7.8 S wild-type NADPH nitrate reductase via a cofactor subunit – cnx.

The cnx is a molybdenum-binding protein of 10 000 – 20 000 daltons. It is thermolabile, stabilized by ionic molybdate, and dissociable by treatment at pH 2–3 from plant and bacterial nitrate reductases as well as from mammalian xanthine, aldehyde and sulfite oxidases [7].

The regulation of nitrate reductase is thought to be controlled by the dehydrogenase locus either via a reversible inactivation by endogenous cyanide with a reductant and reactivation by endogenous dehydrogenase oxidation [8], or inactivation by reduction of the molybdenum in the enzyme from the oxidation state of V to state IV or III.

The redox state is controlled by the ratio of NAD(P)H to NAD(P)$^+$ [1,9].

6.1.2 <u>Nitrite reductase</u>

Nitrite reductase, which catalyzes the reduction of nitrite to ammonia, has been isolated from bacteria, algae, fungi and plants. The enzyme contains iron, has a molecular weight of $0.6-1.2 \times 10^5$ daltons (<u>E. coli</u> and yeast enzymes are larger at $3.5-6.7 \times 10^5$ daltons), and a K_m for nitrite of 0.005–0.07 mmol/l [1].

Plant and algal enzymes are generally specific for single electron donors (e.g. ferredoxin, methyl viologen) and do not utilize NAD(P)H [10]. In yeast and bacteria, the enzyme is NAD(P)H-specific and flavin-dependent [11]. <u>E. coli</u> has two nitrite reductases: one is specific for NADH and unable to reduce sulfite, the other is specific for NADPH and can reduce sulfite. During anaerobic growth in the presence of nitrite, NADPH-specific nitrite reductase is induced, together with a low potential c-type cytochrome (cytochrome c_{552}) that can be reoxidized by nitrite. The metabolic function of cytochrome c_{552} is as yet unclear [12]. Plant nitrite reductases do not reduce sulfite [13].

Most nitrite reductases reduce hydroxylamine [14], although K_m for hydroxylamine is usually about 10 times higher than that for nitrite. Furthermore, the reduction of hydroxylamine is inhibited by nitrite (but not vice versa). Pure preparations of nitrite reductase are unable to reduce hyponitrite or nitric oxide [10]. These data, and the fact that maximum yields of ammonia are produced from nitrite (by pure enzyme), suggest that

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once combined with nitrite, the enzyme may assume a configuration such that exchange between bound and free hydroxylamine does not occur. Regulation of the enzyme is controlled by the redox potential of the cell [15].

6.1.3 Nitrogen reduction in mammalian tissues

Complete reduction of nitrate to ammonia has not been described for mammalian systems, but partial reactions have been shown. For example, nitrate reductase activity has been demonstrated in various rat tissues [16]. Although the enzyme has not been purified, its properties are comparable to those of xanthine oxidase. Xanthine oxidase, as well as aldehyde oxidase (but not sulfite oxidase), can reduce nitrate to nitrite [17]. Interestingly these enzymes share a common interchangeable factor, cnx (molybdenum-binding protein), with microbial nitrate reductase. Both xanthine oxidase and aldehyde oxidase have a molecular weight of 300 000 daltons. They consist of two equal subunits, each containing one atom of molybdenum, one molecule of flavin adenine dinucleotide, and four nonhaem iron-sulfur groups [18].

Also involved in reduction of nitrogenous compounds is the mixed-function oxidase system cytochrome P_{450}. Hepatic microsomal cytochrome P_{450} catalyzes the reduction of tertiary amine oxides to their corresponding amines [19]. The reduction is NADPH- and FMN-dependent and is strongly inhibited by carbon monoxide. Xanthine oxidase enhances nitrogen reduction in the presence of hypoxanthine, probably indirectly, through the reduction of FMN, which could then be reoxidized by the amine oxide. Another partial reduction reaction was discovered when hydroxylamine was incubated with rat liver mitochrondria and NADPH: the hydroxylamine was reduced to ammonia [20].

Apparently, the major systems for oxidizing various carbon compounds are involved in the reduction of nitrogenous compounds. Most of the above studies, however, have been done with regard to specific drug metabolism. Thus, similar experiments with nitrite have not been carried out, and the possibility of nitrate and nitrite assimilation in mammalian tissues has not been investigated since the early part of this century [21].

A dissimilatory mechanism has been suggested for nitrite reduction by bovine heart cytochrome c [22]. A scheme involving cyclic turnover of cytochrome c was suggested to yield nitrogen oxide from nitrite and an electron donor, which is similar to the Pseudomonas aeruginosa dissimilatory nitrite reductase. These mechanisms may function in muscle: nitric oxide has been identified as a product of the anaerobic incubation of fresh pig muscle with nitrite at pH 6.0 [23].

Since nitrate and nitrite reductases are found both in nitrifying and denitrifying bacteria [2], the postulation can be made that mammalian tissues would carry out nitrite assimilation as well as dissimilation.

In conclusion, the enzyme units necessary for conversion of nitrate to ammonia are apparently present in mammalian cells.

6.2 Endogenous nitrate biosynthesis

Although the origin of excess urinary nitrate over nitrate ingestion was first thought attributable to microbial synthesis in the intestinal tract [24], evidence has now shown conclusively that intestinal microorganisms are not responsible for excess urinary nitrate. This conclusion is based on nitrate balance studies in conventional and germfree rats [25,26]. Additional evidence for nitrate biosynthesis in humans is based on long-term (84 days) nitrate balance studies of healthy young men, which showed that the amount of nitrate excreted in urine was on the average four-fold greater than the amount ingested over the entire 84-day experimental period [27]. This observation ruled out the hypothesis that excess urinary nitrate resulted from slow washout of nitrate stored in the body. Furthermore, the use of ^{15}N-labelled nitrate showed that the source of excess nitrate in urine was the endogenous biosynthesis of nitrate, rather than the emptying of a body pool [27,28]. The daily endogenous biosynthesis of nitrate in humans is estimated to be about 1 mmol/day [28].

Wagner et al. [29] recently investigated the mechanism behind the endogenous synthesis of nitrate in mammals using stable isotopes [29]. The oxidation of ammonia to nitrate by microorganisms is well known to be a continuous process of the nitrogen cycle. Thus, to see if ammonia could be converted to nitrate in mammals, Wagner et al. [29] investigated whether isotopically labelled ammonia administered to rats appeared as labelled nitrate in urine. The experiments corroborated the hypothesis.

Effect of inflammation on nitrate biosynthesis: During the course of nitrate balance studies in adults, Wagner & Tannenbaum [30] observed an unexpected stimulation of nitrate biosynthesis in one subject who developed a fever and nonspecific intestinal diarrhoea. Urinary nitrate excretion increased nine-fold during the illness compared with that before the symptoms appeared. A similar elevation of urinary nitrate excretion has been noted by Hegesh & Shiloah [31] in infants suffering from diarrhoea. Therefore, the possibility was explored that rats exposed to

E. coli lipopolysaccharide (LPS), which can induce fever and diarrhoea, have enhanced nitrate biosynthesis [29].

Fever induced by E. coli LPS endotoxin had a striking effect on urinary nitrate excretion. Urinary nitrate excretion increased nine-fold (45 +/- 6.3 umol/day) during the first day of the fever compared with an average nitrate excretion of 5.2 +/- 0.13 umol/day during the week preceding LPS administration. As the fever subsided, nitrate excretion decreased, and 5 days after injection, nitrate levels returned to initial values. Control rats injected with 0.9% saline solution showed no increase in nitrate excretion.

The enhanced urinary excretion of nitrate during LPS administration was shown to result from an increased rate of nitrate synthesis using ^{15}N-ammonium acetate as a nitrogen donor [29]. The increased excretion of nitrate after LPS administration was accompanied by increased incorporation of ^{15}N-ammonia into nitrate-nitrogen. The significantly greater amounts of ^{15}N-nitrate produced from labelled ammonia with LPS treatment suggest that more ammonia nitrogen is shunted into nitrate biosynthesis during LPS treatment.

Two other types of inflammatory state produced changes in nitrate biosynthesis. A carrageenan-induced inflammation produced a greater than two-fold increase in nitrate synthesis. Nitrate levels returned to baseline values after 3 days. On the other hand, a turpentine-induced inflammation produced a delayed pattern of enhancement of nitrate biosynthesis. Nitrate levels did not significantly increase during the first 24 hours after turpentine administration but thereafter increased approximately three-fold. This is further evidence for induction of nitrate synthesis by an activated reticuloendothelial system.

These findings support the hypothesis that activation of the reticuloendothelial system significantly increases nitrate biosynthesis. One possible mechanism is that the increased nitrate synthesis may have been due to an increased generation of reactive oxygen species capable of oxidizing reduced nitrogen compounds to nitrate. Potential oxidizing species, derived from an activated immune system, that could oxidize reduced nitrogen compounds to nitrate include superoxide, hydrogen peroxide, hydroxyl radical, and singlet oxygen. Further experiments designed to block the increased synthesis of reactive oxygen species after LPS administration will be necessary to test this hypothesis.

6.3 Metabolism and distribution of nitrite and nitrate in the body

Nitrate and nitrite are both formed and destroyed in vivo. These processes may be the metabolic actions of microflora normally or abnormally resident in the animal host, or may result from the animal's own metabolism. For example, nitrate is reduced to nitrite by normal microflora in the mouth, microorganisms that colonize the abnormally hypochlorhydric or achlorhydric stomach, and flora that inhabit the infected urinary tract or bladder.

Nitrate may also be reduced by mammalian enzymes in vitro. Nitrate is formed both from nitrite via oxyhaemoglobin and more-reduced nitrogen compounds by mammalian systems.

This section discusses the metabolism, distribution and clearance of nitrate and nitrite in the mammalian body.

6.3.1 Reduction of nitrate to nitrite

People ingest from tens to several hundred milligrammes of sodium nitrate per day from food and water [32-34]. As nitrate levels in saliva rise to reflect this ingestion, levels of salivary nitrite also increase due to the action of reductase systems of the oral microflora [35-41]. Both nitrate and nitrite levels in saliva peak from 2-4 hours after ingestion of nitrate-rich food or drink, reaching levels of up to several hundred milligrammes per litre depending on the ingested amount and concentration of nitrate [39-41].

The kinetics are governed by the absorption, distribution and secretion of nitrate. The return of basal nitrite levels requires several hours, and the contribution of nitrite by the saliva to the rest of the gastrointestinal system may be significant. Ishidate et al. [38] estimate that saliva might contribute 50-70 mg of nitrite after a nitrate-rich meal; Spiegelhalder et al. [41] suggest that 40 mg of nitrite could be formed within 5 hours after eating lettuce or other nitrate-rich vegetables or juices. Considerable variation in salivary nitrite production among humans and monkeys has been noted [42,43]. Lowenfels et al. [44] reported that oesophageal cancer patients as a group were no different from healthy controls in their ability to reduce nitrate to salivary nitrite. Fasting levels of salivary nitrite average 5-10 mg [41,45] and do not differ between smokers and nonsmokers [46].

Microbial reduction of nitrite is not confined to the oral cavity. The results of Klein et al. [47] suggest that microfloral production of nitrite continues in the oesophagus, and several investigators have shown that stomachs with reduced acidity and therefore favourable environments for microbial

growth are likewise conducive to nitrite production. Thus, Sander & Seif [48] showed that a fasting gastric pH of 4 or greater allowed nitrate reductions in patients' stomachs.

Ruddell et al. [49] demonstrated the inverse relationship between nitrite and hydrogen ion concentration in the stomach of normal individuals, ulcer patients, gastric cancer patients, and otherwise normal individuals with hypochlorhydria. Individuals with abnormally low, fasting gastric acidity had average gastric concentrations of nitrite of 30-40 umol/l (1-2 mg/l). The same workers [50] also reported average nitrite concentrations of 120 umol/l in the achlorhydric stomachs of pernicious anaemia patients. In normally acidic stomachs, nitrite averaged 2-3 umol/l. Tannenbaum and co-workers [51] also found considerably greater than normal concentrations of nitrite in the stomachs of individuals whose fasting gastric pH was 5 or higher. Jones et al. [52] reported levels of 10-30 umol/l in stomachs of post-partial gastrectomy patients displaying dysplasia. Schlag et al. [53] also found elevated nitrite levels in Billroth II resected stomachs. In general, the above-cited authors hypothesize that the increased gastric cancer risk of these patients with pernicious anaemia or resected stomachs might involve their increased levels of gastric nitrite.

In 1914, Cruickshank & Moyers [54] reported on the presence and significance of nitrite in urine. They assayed 600 urine samples for nitrite and concluded that nitrite was not present in normal urine from healthy individuals; in cases where nitrite was present, Gram-negative bacteria were also present. Since then, many investigators have tested the efficacy of diagnosing urinary tract infections using nitrite test strips [55-59]. However, while false-positive results are very rare, the rather sizeable proportion of false-negative (i.e. nitrite-free urines that belie infections) appears to limit the usefulness of this screening test. Several other investigators have studied urinary nitrite and urinary tract infections with respect to in situ formation of nitrosamines [60-63].

In general, body sites containing both microflora and nitrate will generate nitrite. The small intestine and lower parts of the gastrointestinal tract are, of course, rife with microorganisms, but nitrate and nitrite have not been found generally in the lower gut or in faeces [64].

6.3.2   Distribution and clearance of nitrate

Animals dosed with nitrate excrete the bulk of it into urine within a day [65-67]. Although varying and dose-dependent recoveries of nitrate in urine have been reported, the amount of nitrate excreted generally reflects the amount of nitrate

ingested plus the endogenous component [68-72]. People on various diets have urinary nitrate concentrations of about 1 mmol/l and plasma nitrate levels of approximately 0.2 mmol/l [28], indicating that a five-fold concentration of nitrate is typically achieved by the kidneys. In an experiment designed to mimic the effects of repeated nitrate ingestion with meals and water, Tannenbaum et al. [73] found diurnal patterns of nitrate excretion in urine and nitrate and nitrite levels in saliva.

A one-compartment pharmacokinetic model was used to analyse plasma and urine data obtained in one study [28]. Nitrate entry into the body can occur by two routes: namely, dietary intake and endogenous synthesis. Nitrate is removed by urinary excretion and reaction to reduced forms of nitrogen. When nitrate inputs to the body are taken to be constant and removal processes are assumed to be first order in nitrate concentration, a one-compartment pharmacokinetic model leads to the following equation to describe the plasma nitrate concentrations:

$$V_D \cdot \frac{dC}{dt} = R - k_T \cdot V_D \cdot C$$

where V_D is the volume of distribution in the body, C is the plasma nitrate concentration, R is the net rate of input (primarily endogenous synthesis), and k_T is the total elimination constant (units of inverse time). The solution to this equation is:

$$c(t) = \frac{R}{k_T V_D} + C_0 e^{-k_T t}$$

where C_0 is equal to the size of the dose divided by V_D. $R/k_T V_D$ is seen to be the steady-state plasma nitrate concentration C_{ss}. The above equation predicts that plotting $[C(t) - C_{ss}]$ versus time semilogarithmically should yield a straight line with slope $-k_T$ and intercept C_0.

From the data in Table 4, C_{ss} appears to have a mean value of about 0.03 mmol/l. This value was subtracted from the mean plasma concentration following ingestion of the nitrate dose and plotted versus time on semilog coordinates. Removal of nitrate from the body was found to be primarily first order in plasma nitrate concentration (data not shown), and k_T to be 0.14/h, corresponding to a half-life for nitrate in the body of 5 hours. C_0 was determined by extrapolating the semilog plot to time 0 and was found to be 0.135 mmol/l, indicating a volume

of distribution for nitrate of 21.1 litres (V_D = dose/C_0).
Since the mean weight of all 12 subjects was 71.4 kg, the
nitrate space in humans is about 30% of body weight. The data
of Ellen et al. [74], who administered a dose of up to 130 mmol
of nitrate, also show an exponential decay in plasma nitrate
concentrations after ingestion and suggest a similar volume of
distribution of about 30% of body weight.

The total clearance of nitrate from the body can be
estimated by multiplying k_T and V_D, which yields 2.9 1/h for
subjects in this study. Urinary clearance was calculated by
dividing the average rate of urinary excretion by the log mean
plasma nitrate concentration for each urine collection period,
yielding a mean value of 1.6 1/h. This ratio of renal to total
clearance (1.6:2.9), determined from the data for total nitrate
($^{14+15}$N), provides an independent prediction of the fraction
of nitrate presented to the body which will appear unmetabolized
in urine. The urinary clearance was calculated to be 55% of
total clearance, which is in good agreement with the recovery
of 60% of the administered $^{15}NO_3^-$ in urine as nitrate.

6.3.3 Nitrate and nitrite in the oral cavity and stomach

The possible health risks posed by nitrate are due to
endogenous processes which convert nitrate to nitrite. The
pharmacokinetic model for nitrate which has been presented lays
much of the groundwork for developing a model to describe these
processes. As the oral cavity is an important site of nitrite
generation from nitrate and the stomach is a likely site for
N-nitrosation by nitrite, a pharmacokinetic model which attempts
to relate endogenous N-nitroso compound synthesis with dietary
nitrate must incorporate compartments representing these
regions.

If the oral cavity is viewed as a well-mixed compartment,
the nitrate and nitrite conservation equations may be written as:

$$V_0 \frac{dY_0}{dt} = Q_S [(S:P)Y_P - Y_0] - R_0 V_0$$

$$V_0 \frac{dZ_0}{dt} = R_0 V_0 - R_0' V_0 - Q_S Z_0$$

where V_0 is the volume of saliva in the mouth, Q_S is the
saliva flow rate, Y_0 and Z_0 are the nitrate and nitrite
concentrations of the saliva within the oral cavity,
respectively, R_0 is the volume-specific rate of oral bacterial

Table 4.   Nitrate levels in plasma (P) and nitrate and nitrite levels in saliva (S)
after a 3.5 mmol dose of nitrate[a]

Time after dose (h)	Plasma nitrate (mM)	Salivary nitrate (mM)	Salivary nitrite (mM)	S:P ratio	Salivary nitrite/NO_3^-
0	0.03 +/- 0.01[b]	0.20 +/- 0.09	0.09 +/- 0.04	13 +/- 9	0.42 +/- 0.18
0.5		2.3 +/- 0.76	0.53 +/- 0.22		0.23 +/- 0.10
1	0.17 +/- 0.03	2.5 +/- 0.77	0.48 +/- 0.24	20 +/- 7	0.19 +/- 0.08
2		2.3 +/- 0.87	0.52 +/- 0.27		0.23 +/- 0.11
3	0.14 +/- 0.02	1.9 +/- 0.78	0.49 +/- 0.20	20 +/- 7	0.30 +/- 0.14
6	0.10 +/- 0.01	0.81 +/- 0.18	0.29 +/- 0.16	13 +/- 6	0.38 +/- 0.16
12	0.06 +/- 0.01	0.38 +/- 0.20	0.15 +/- 0.06	12 +/- 7	0.38 +/- 0.17
24	0.04 +/- 0.01	0.43 +/- 0.19	0.12 +/- 0.07	15 +/- 5	0.30 +/- 0.15
48	0.03 +/- 0.02	0.28 +/- 0.25	0.10 +/- 0.08	12 +/- 5	0.42 +/- 0.25

[a]   Twelve subjects on a low ascorbic acid diet (60 mg/day). Dietary nitrate was 0.15 mmol/day.

[b]   Mean +/- S.D.

reduction of nitrate to nitrite, and R_0' is that for further reduction of nitrite. Since S:P is the saliva-to-plasma ratio, (S:P)Y_p is the nitrate concentration of saliva as it enters the mouth (ductal saliva). This should be viewed as an average of the nitrate concentrations generated by the individual pairs of glands [75,76], weighted according to their relative flow rates.

Based on simultaneous measurements of plasma and mixed saliva, Wagner et al. [28] argue that, in addition to effecting the conversion of nitrate to nitrite, the oral microflora further reduces nitrite. If true, the oral cavity may be another important site of metabolic nitrate losses from the body. Mere conversion of nitrate to nitrite cannot be considered a metabolic loss of nitrate because nitrite is readily oxidized back to nitrate in the blood. However, if nitrite is reduced to NO, N_2O, or N_2 gas and exhaled, or if it is reduced to aqueous species not readily oxidized back to nitrate, this constitutes removal of nitrate from the body. Quantification of nitrite reduction in the mouth requires a measure of the nitrate concentration of ductal saliva in addition to the nitrate and nitrite concentrations of mixed saliva. When simultaneous plasma, mixed saliva, and ductal saliva data are available, the two nitrite conservation equations given above can be incorporated into the set of equations for the other body compartments and solved to determine the parameters in the expressions for R_0 and R_0'. The data of Wagner et al. [28] indicate that a saturable expression for R_0, such as Michaelis-Menten, is necessary to account for the observed nitrate and nitrite contents of mixed saliva.

In the normal stomach, where bacterial levels are low, nitrate is stable, meaning that salivary nitrite is the predominant source of gastric nitrite. In the hypoacidic stomach, however, bacterial reduction of nitrate may be the major source of nitrite. Based on studies of iodide and pertechnetate [77], nitrate enrichment of gastric juice to a degree similar to that of saliva seems likely.

The pK_a of nitrous acid is 3.4; in the acidic stomach, most of the nitrite is present in the protonated form or as nitrous anhydride (N_2O_3) which can form as follows:

$$H^+ + NO_2^- \rightleftharpoons HNO_2$$

$$H^+ + NO_2^- + HNO_2 \rightleftharpoons N_2O_3 + H_2O$$
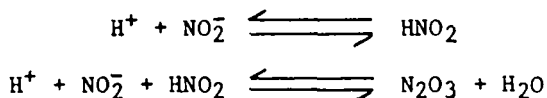
In the absence of nitrosation promoters, the reaction kinetics for nitrosamine formation in simple systems are second order with respect to nitrite concentration, indicating that

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nitrous anhydride is the major nitrosating agent under these conditions [78,79]. When high concentrations of promoters such as thiocyanate are present, the rate of nitrosation is greatly increased and the kinetics are first order in nitrite [78]. In both cases, the reaction rates are strongly influenced by the pH of the system [78,79]. Still unknown, however, is how much nitrite loss by reaction in the complex milieu of the stomach is due to nitrosation and how much is due to other reaction mechanisms. Furthermore, in addition to loss by chemical reaction, nitrite can cross the gastric mucosa and enter the bloodstream. The formation of the neutral species nitrous acid [80] and nitrous anhydride [81] has been attributed to this transfer.

6.4 Formation and occurrence of nitrosamines

The literature on nitrosamine formation and occurrence is extensive and cannot be adequately reviewed in this report. The reader is referred to several important reviews which have recently appeared [82-84]. The following is a synopsis of available knowledge.

6.4.1 Environmental contamination

Systematic analyses of the environment have been made in several countries by the thermal energy analyzer (TEA) method. Probably the most comprehensive survey of products in any one country has been made by Preussmann and his colleagues in Heidelberg. They have identified many types of contaminated products and have helped the industries involved to eliminate the source of contamination [85]. Among the many sources of nitrosamines, the following are particularly significant.

(a) Tobacco products

Cigarettes, chewing tobacco and snuff are probably a major external source of human exposure; nonsmokers are exposed by way of sidestream smoke from cigarettes [86]. Cigarette smoke contains so many carcinogens that separating the effects of each is difficult. A more intensive investigation of betel-nut chewing might yield additional evidence linking nitrosamines and cancer.

(b) Cured meat products and malt beverages

Low levels (< 10 ppb) of nitrosamines are still found in some products, but new technological approaches have effected

reductions greater than 90% of the levels that existed when contamination was discovered.

(c) Cosmetics

The alkanolamine content of these products causes absorption of nitrogen oxides, and a nitrosating reaction may take place in a non-aqueous environment [87]. Diethanolnitrosamine is carcinogenic in rats, but its toxicological significance for humans is not known. Other nonvolatile nitrosamines may be present, but few data are available.

(d) Corrosion inhibitors

Amine salts of nitrite lead to the formation of various nitrosamines, primarily diethanolnitrosamine. As stated above, the toxicological significance of this compound is unknown, but an opportunity exists to conduct epidemiological studies in the industries involved.

(e) Rubber products

A wide variety of rubber products and materials may be contaminated. The latest finding was of contaminated rubber nipples for infant feeding bottles [88]. The extent and nature of human exposure would be difficult to estimate, and no epidemiological studies have been conducted.

6.4.2 Human exposure

Environmental contamination may lead to significant exposure of humans to nitrosamines, but isolation of a single contamination source which can be identified with a single human disease is difficult. In most cases, contamination levels have been reduced after their discovery, making epidemiological investigation difficult. Continued diligence in reducing exposure levels may lead eventually to a downward trend in some diseases that can be analysed retrospectively.

In considering the overall exposure of an individual, account must be taken of both lifestyle and workplace, as well as endogenous factors. Because the amount of nitrosamines to which an individual is exposed daily cannot be measured, estimates of exposure are usually made by calculating averages of different exposures. The individual at greatest risk is obviously the person whose combination of lifestyle and vocation lead to the greatest accumulation of exposure sources [89].

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Attempts have been made to estimate the daily exposure to
nitrosamines from a variety of sources. The estimate for the
United Kingdom is given in Table 5, the Netherlands in Table 6,
the Federal Republic of Germany in Table 7 and the United States
in Table 8.

6.4.3 Endogenous formation

Many workers have considered the impact of endogenous
formation of N-nitroso compounds from the precursors nitrate or
nitrite and various nitrogen compounds. That this reaction
could take place in the human stomach was demonstrated 15 years
ago by Sander [90], and the concept has been extended and
characterized in laboratory animals by many other workers [91].
Not until 1981, however, was the quantitative formation of a
nitrosamine in a human demonstrated [92]. This was accomplished
by feeding nitrate and proline and measuring the excreted
N-nitrosoproline (NPro) in the urine. Magee and co-workers have
shown that this compound is not a carcinogen and is not

Table 5. Estimated daily intake of volatile nitrosamines in
food consumed in the United Kingdom[a]

NOTE: Several numbers have been rounded off to two
significant figures.

Food	Food consumption, g/person/day	Total nitrosamine intake ug/person/day
Cured meats	49	0.43
Fish	20	0.01
Cheese	14	0.01
All other foods	1400	0.08
Total	1500	0.53

[a] Adapted from Gough et al. [93].

Table 6. N-Nitrosodimethylamine (NDMA) content of various kinds of food in the Netherlands[a]

Food	Number of samples	NDMA content (ug/kg)		Percentage of positive samples[b]
		Mean	Range	
Beer	57	1.2	0[c] - 5.7	72
Whiskey	7	0.3	0 - 0.9	86
Cured meat	38	0.5	0 - 3.6	71
Veal	22	0.1	0 - 0.4	29
Seafood	53	0.4	0 - 2.1	55
Cheese	84	0.1	0 - 1.1	45

[a] Adapted from Stephany & Schuller [94]
[b] Samples with content equal to or higher than limit of detection (0.1 ug/kg).
[c] 0 = Content below limit of determination.

metabolized, indicating that the amount of NPro excreted is the actual amount synthesized [97]. Bartsch & Ohshima [92] have used this approach to demonstrate the efficacy of ascorbic acid and alpha-tocopherol in blocking intragastric nitrosation, giving support to the idea that large doses of these vitamins may be useful when intervening in high-risk populations.

Tannenbaum and co-workers have confirmed and extended the findings on urinary NPro excretion. The major portion of endogenous synthesis appears to occur in the stomach. The amount of NPro formed is a function of exposure to nitrate (nitrite) and proline and the phasing of their appearance in the stomach. If ascorbic acid and alpha-tocopherol were to be completely effective in inhibiting nitrosation, they would have to be present in sufficient concentration in gastric juice at all times: controlled dosage forms would be needed to achieve this. A major unanswered question is what period of the day is most significant for the nitrosation process: during the day when the stomach contains food, during the night when it is empty, or perhaps in some intervening period? The optimum conditions for reaction would be those that had the highest reactant concentrations and a low pH, but no period appears to offer the optimum conditions. Should a blocking agent be taken with a meal or at bedtime? Research in this direction is required.

Table 7. Average daily intake of nitrosamines by males in the Federal Republic of Germany. Calculated from average per capita consumption[a]

Food	Product consumption (g)	Nitrosamine[b]	Nitrosamine intake (ug per capita)
Beer[c]	560	NDMA	0.7
Meat and meat products	210	NDMA	0.1
		NPYR	0.1
		NPIP	0.01
Cheese	30	NDMA	0.01
Others	1500	NDMA	0.2
		NPYR	0.03
Total	2300	NDMA	1.1
		NPYR	0.13

[a] Adapted from Spiegelhalder et al. [95].
[b] Abbreviations for Tables 7 and 8:
  NDMA = N-nitrosodimethylamine
  NDEA = N-nitrosodiethylamine, NMOR = N-nitrosomorpholine
  NPIP = N-nitrosopiperidine, NPYR = N-nitrosopyrrolidine
  NNN = N'-nitrosonornicotine, NAT = N'-nitrosoanatabine
  NAB = N'-nitrosoanabasine,
  NNK = 4(methylnitrosamino)-1-(3-pyridyl)-1-butanone
  NEMA = N-nitrosoethylmethylamine,
  NDELA = N-nitrosodiethanolamine.
[c] NDMA intake is corrected to account for the proportion of sales for different types of beer.

Table 8. Estimated exposure of humans to nitrosamines in the United States

Source of exposure	Nitrosamine[a]	Primary exposure	Concentration	Daily intake (ug/person)
Cigarette smoking[b]	NDEA	Inhalation	1.0 ng/cig)	
	NEMA	Inhalation	0.5 ng/cig)	
	NDMA	Inhalation	6.5 ng/cig)	
	NPYR	Inhalation	7 ng/cig)	17
	NDELA	Inhalation	24 ng/cig)	
	NNN	Inhalation	310 ng/cig)	
	NAT	Inhalation	370 ng/cig)	
	NNK	Inhalation	150 ng/cig)	
Automobile interiors	NDMA)		1.0 ug/m^{3c}	0.50^c
	NMOR)	Inhalation		
	NDEA)		0.35 ug/m^3	0.20^d
Beer	NDMA	Ingestion	2.8 ug/l	0.97
			1.0 ug/l	0.34
Cosmetics	NDELA	Dermal	11 mg/kg	0.41
Cured meat; cooked bacon	NPYR	Ingestion	5 ug/kg	0.17
Scotch whiskey	NDMA	Ingestion	0.97 ug/l	0.03

[a] For abbreviations, see Table 7.
[b] Adapted from [89].
[c] Estimate represents exposure from new automobile interiors. Average nitrosamine concentration was taken from Rounbehler et al. [96]. An assumption of an average daily exposure of approx. 1 h/day was made.
[d] The assumption was made that the average daily exposure is approx. 1 h/day and that it occurs in both new and older automobiles. Since nitrosamine concentration is likely to be lower in older automobiles, the average concentration has been reduced to one third of that in new automobiles.

The amount of NPro excreted is about 10 ug/day in normal healthy adults. No measurements have as yet been reported on subjects possibly prone to increased output due to more favourable reaction conditions or greater exposure to nitrite. More important, as the nature of all the putative carcinogens that might form endogenously is not known, calculating the actual exposure to carcinogens with N-nitroso structure cannot be done. However, the assumption can be made that exposure will probably be at least in the order of several micrograms per day and perhaps considerably more. This estimate is greatly in excess of the exposure from food and beverages but is not necessarily greater than exposure in certain workplace situations.

Intelligent guesses can be made about some types of nitrosamine that might be formed endogenously; they include the derivatives of amines commonly found in body fluids (i.e. dimethylamine, pyrollidine and piperidine). The difficulty of demonstrating the formation of these compounds can be ascribed to the rapidity with which they are metabolized in the body (they have half-lives of about 30 minutes). Identification of nitrosamides, which are unstable both biologically and chemically in the body, is even more difficult. Tannenbaum and co-workers have studied the N-nitroso-conjugated bile acids, N-nitrosoglycocholate and N-nitroso-taurocholate, as nitrosamides which could possibly form in the gastric environment after bile reflux. They have shown that these compounds are potent mutagens both for bacteria and human cells in culture [98] and are also potent carcinogens when given orally to rats. The results are not sufficiently well analysed to permit discussion of organ specificity, but if these compounds are carcinogenic in the stomach or intestinal tract, this finding would be of great etiological significance.

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7.   ADVERSE HEALTH EFFECTS OF NITRATE INGESTION

Exposure to high doses of nitrate, nitrite, and N-nitroso compounds has been associated with a variety of adverse health effects in humans or other species. In a comprehensive review in 1981, the National Academy of Sciences summarized this information and described the most prevalent adverse effects [1].

Nitrate itself is relatively nontoxic, but when ingested in food or water, it may be reduced to nitrite by bacteria in the mouth and gut; nitrite is a powerful oxidizing agent, capable of converting haemoglobin in the blood to methaemoglobin. Nitrite may also react in vivo with nitrosatable substrates in certain foods to form N-nitroso compounds. Whereas no evidence indicates that either nitrates or nitrites are themselves carcinogenic, many N-nitroso compounds have been found to be carcinogenic when administered to laboratory animals; as yet, they have not been incriminated definitely as the cause of any human cancer [2].

The principal sources of dietary nitrate are drinking-water, vegetables and cured meat products. When the concentration of waterborne nitrate is high, drinking-water contributes substantially to total nitrate intake [3], and the potential for nitrite, methaemoglobin and N-nitroso compound formation may be increased.

7.1   Methaemoglobinaemia

Methaemoglobinaemia is not a notifiable disease in many European countries, making its true incidence unknown. Morbidity and mortality statistics are often not helpful because methaemoglobinaemia is frequently classified with other rare and unspecified diseases of the blood and blood-forming organs.

7.1.1 Methaemoglobinaemia in infancy

That excessive quantities of nitrate in drinking-water present a health risk to young, artificially fed babies has been recognized since the first cases of cyanosis due to methaemoglobinaemia were described by Comly in 1945 [4]. Low gastric acidity in infants permits the growth of nitrate-reducing bacteria in the upper gastrointestinal tract, allowing ingested nitrate to be reduced to nitrite. The effect is enhanced in the presence of infection causing diarrhoea. On absorption, the nitrite combines with haemoglobin to form methaemoglobin, which cannot transport oxygen. The infant suffers from cellular anoxia, manifest as clinical cyanosis,

when approximately 10% of total haemoglobin has been converted
to methaemoglobin [5]. Methaemoglobin-reducing enzymes work to
restore the status quo and normally only 1-2% of total
haemoglobin is present as methaemoglobin. Intravenous methylene
blue and ascorbic acid have proved useful in treating
methaemoglobinaemia, though survival is rare when more than half
of the total haemoglobin has been converted to methaemoglobin.

Several other factors render infants under 3 months of age
particularly susceptible to development of methaemoglobinaemia
from nitrate in drinking-water. In addition to their low
gastric acidity which allows coliform organisms to flourish,
infants have a higher fluid intake in relation to their body
weight than adults. Fetal haemoglobin, which predominates in
the newborn, is more readily oxidized by nitrite than adult
haemoglobin, and the enzyme systems responsible for
methaemoglobin reduction are not fully developed at birth.
Nitrate may be concentrated by repeated boiling of water for
feedings, and bacterial contamination of the water itself or the
dried milk powder [6] may increase the potential for nitrate
reduction. Early weaning onto nitrate-rich vegetables, such as
spinach, and medication with drugs prescribed for infantile
diarrhoea, such as bismuth subnitrate, may provide additional
sources of nitrate. Some evidence indicates that vitamin C may
play a role in preventing the disease [7].

A novel approach to risk estimation of nitrate-induced
methaemoglobinaemia is given in Annex 1.

Since 1945, some 2000 cases of methaemoglobinaemia have
been reported in the world literature [7], with a case fatality
of about 8%. The WHO standards for nitrate in drinking-water
[8,9] are based largely on an analysis of reported cases in
relation to the level of nitrate in the water associated, with
each case. The survey results of Walton in the United States in
1951 [10], Sattelmacher in 14 countries in 1962 [11], and Simon
et al. in Germany in 1964 [12] suggest that most cases of
methaemoglobinaemia have occurred when nitrate levels have
exceeded 22.6 mg/1. Cases where nitrate levels are less than
11.3 mg/1 are rare, the surveys reporting 0.0, 3.0 and 4.4%,
respectively, when nitrate levels were below this level.
However, in these surveys the nitrate concentrations were
unknown in 32%, 56% and 67% of cases, respectively. Even
available data on nitrate levels may be misleading when water
samples for analysis are obtained weeks or months after the
acute illness, during which time the nitrate concentration may
have changed considerably. Most cases of methaemoglobinaemia
reported in the world literature have been associated with
private and often bacterially contaminated wells, rather than
public water supplies [5].

(a) Hungary

Since 1968, methaemoglobinaemia has been a notifiable disease in Hungary. The following unpublished data were made available by S. Deak.

In the first five years after 1968, a total of 883 cases was reported, the number ranging from 155-189 per year. During 1975-1977, 190 cases of methaemoglobinaemia were reported in four counties with a total population approaching 1.7 million: 94% of the cases were 3 months old or younger. The death rate was 3.2%. The nitrate level in the drinking-water exceeded 22.6 mg/l in 92% of the recorded cases, while in the remaining 8%, it was between 9.0-22.6 mg/l.

From 1976-1982, 1353 cases of methaemoglobinaemia, including 21 deaths, were reported in Hungary. The highest number (293 cases with 7 deaths) was reported in 1977. The increasing number of cases of methaemoglobinaemia led to a survey to obtain information about the nitrate level in villages not supplied with low-nitrate, piped drinking-water. In approximately 25% of the villages which had water supplied from individually dug wells, the nitrate level exceeded the Hungarian standard of 9.0 mg/l. In 7.5% of the villages, the nitrate content of available drinking-water was in excess of 22.6 mg/l and about 9000 infants were at risk.

Measures were taken to supply the population with drinking-water of low nitrate content. These measures have resulted in a definite decrease in the number of cases of methaemoglobinaemia each year since 1979. Certain counties have had no or very few cases of methaemoglobinaemia registered; the water source of these counties is mainly deep-well water, which is almost free of nitrate. The highest number of cases occurred in two counties where distributed water in the villages is unusual and where the nitrate level in the groundwater is high.

More detailed information is available for the year 1982. The 96 cases recorded in this year comprised 50 males and 46 females, and 92% of the patients were 3 months old or younger. The nitrate level of the drinking-water varied between 14.7-187 mg/l. However, in 7% of the cases the level was between 9.2-22.6 mg/l. All registered cases were observed in connection with privately dug wells. All patients received hospital treatment.

(b) United Kingdom

Ten cases of methaemoglobinaemia have been reported in the United Kingdom in the last 30 years, with one death [13]. As a consequence of the drought in 1976, water containing between

11.3-22.6 milligrams of NO_3-N per litre was used in some areas. Low-nitrate bottled water was provided for infant feeding where necessary, as has been the custom in the United Kingdom since 1950 when the first cases of methaemoglobinaemia were 'reported in East Anglia. All health authorities were alerted to the potential danger in 1976 and asked to notify any cases of infantile methaemoglobinaemia to the Department of Health; no cases were reported [14].

(c) Other European countries

During the last ten years, no or only very occasional cases of methaemoglobinaemia associated with nitrate in drinking-water have been reported in Denmark, the Netherlands and Sweden: such cases have been related to water from private wells.

(d) Subclinical effects of nitrates in infants

In contrast to the overt clinical cases, rather less is known about the subclinical effects of using high-nitrate water for infant feeding. A study by Shearer et al. [15] of 487 methaemoglobin levels in 256 Californian infants from birth through 6 months showed that even healthy babies not exposed to excessive nitrate levels in diets have higher levels when young. Twenty-one samples (4.3%) had more than 4% of methaemoglobin, the highest levels being found in babies with diarrhoea or respiratory illness. No association was found between the frequency of elevated methaemoglobin levels and well-water nitrate levels which occasionally exceeded 10 mg/l. However, methaemoglobin levels above 4% were more prevalent when nitrate intake, calculated from the history of ingestion and the measured levels in water and formulae, exceeded 5 milligrams per day of oxidized nitrogen. Bacterial contamination of more than 60% of formulae may have contributed to these elevated levels.

Shuval & Gruener [16] looked for evidence of chronic subclinical disease in 400 infants exposed to drinking-water nitrate levels of 10-20 mg/l in the Gaza area in Israel. Although no clinical cases of methaemoglobinaemia were detected, a significant increase in methaemoglobin levels was apparent even in the group of infants exposed to water (consumed mainly as powdered milk formula) containing 10 to 12.4 milligrams of NO_3-N per litre.

More recently, Super et al. [17] studied the prevalence of subclinical methaemoglobinaemia and its possible morbidity in a group of 486 Southwest African/Namibian infants. The geographical area studied was divided into low and high nitrate regions according to whether the well-water nitrate

concentration was below or above 4.5 mg/l. A strong association was found between nitrate region and methaemoglobin levels: in the high-nitrate region, 33% of infants had more than 3% of methaemoglobin while in the low-nitrate region, only 13% exceeded this level. Actual nitrate intake was also closely correlated with methaemoglobin levels. Unlike the previous studies, no association with age was demonstrated, a likely explanation being the rarity of vitamin C administration in this population compared to the Californian and Israeli infants. Where vitamin C was consumed regularly, it was associated with significantly lower methaemoglobin levels. A history of diarrhoea and/or respiratory infection or physical or developmental delay could not be correlated with nitrate intake or methaemoglobin level, but deaths in infancy were more common in the high-nitrate region.

7.1.2 Methaemoglobinaemia in childhood

Several studies in the USSR have suggested that elevated methaemoglobin levels from ingested nitrate may not be confined to young infants. Levels up to 7% in schoolchildren have been associated with drinking-water nitrate levels of 23-204 mg/l [18]. Petukhov & Ivanov [19] also described slowing of conditioned motor reflexes in response to auditory and visual stimuli in 39 Russian children whose drinking-water contained 23.7 milligrams of nitrate per litre. Their reflexes were compared with those of a group of children whose drinking-water contained only 1.8 mg/l. The concentration of methaemoglobin did not exceed normal limits in the schoolchildren who drank low-nitrate water, whereas the children exposed to high-nitrate water had an average of 5.3% of methaemoglobin in their blood. Methaemoglobinaemia with less than about 10% of methaemoglobin has been generally regarded as clinically insignificant [5]. Therefore, this report of a measurable effect on the central nervous system with only 5% reduction in oxygen-carrying capacity warrants further study.

By contrast, an epidemiological study of 102 American children aged 1-8 years in Illinois [18] did not show that ingestion of water with a nitrate concentration of 22-111 mg/l was related to increasing methaemoglobin levels. Neither was a trend indicated towards higher levels of methaemoglobin in young children using wells with greater bacterial contamination.

7.1.3 Methaemoglobinaemia in other predisposed groups

Several other categories of individuals with altered physiological states or either with hereditary or acquired

disease may also be predisposed to the development of methaemoglobinaemia [1]. These include pregnant women, individuals with glucose-6-phosphate dehydrogenase deficiency, adults with reduced gastric acidity, and rare groups with a hereditary lack of methaemoglobin reductase activity in their red blood cells.

7.1.4 Conclusions about methaemoglobinaemia

High levels of nitrate in drinking-water have been associated with infantile methaemoglobinaemia. Most cases have been associated with drinking-water with nitrate levels exceeding 25 mg/l, but in some cases the nitrate level was only 10-20 mg/l. In most cases the drinking-water consumed by the affected infants was derived from shallow wells and was almost certainly contaminated with bacteria. The nitrate level in water is only one factor in the etiology of methaemoglobinaemia, and large variations in individual susceptibility occur. The significance, if any, of subclinical levels of methaemoglobin in infancy and childhood remains unclear.

7.2 Cancer, with emphasis on gastric cancer

7.2.1 Epidemiological studies of carcinogenicity

The amount of N-nitroso compounds which can be formed in vivo depends in part on the availability of nitrite, which is itself dependent on the availability of nitrate, the presence of a microbial population with nitrate reductase activity, and conditions favourable to chemical nitrosation [20]. Therefore, if endogenously formed N-nitroso compounds are important in human cancer, populations ingesting larger amounts of nitrate might be expected to have a higher incidence of cancer of the relevant target organ. With the exception of studies of oesophageal cancer in Iran and China and a few studies which have considered cancer risk in general, most epidemiological investigations have examined this hypothesis in relation to gastric cancer [1].

In 1979, the United Kingdom Royal Commission on Environmental Pollution [14] and independent researchers including Fraser et al. [21], looked at the results of epidemiological studies available at that time and concluded that no evidence unambiguously associated nitrates, nitrites or N-nitroso compounds with cancer of any organ in humans. The reviews took into account the results of studies relating gastric cancer risk to nitrate fertilizer use in Chile [22-25],

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and to waterborne nitrate levels in Colombia [26] and the Nottinghamshire mining town of Worksop in the United Kingdom [27]. In Chile, nitrate fertilizer application was used to estimate population exposure to nitrate, whereas in Colombia and Worksop, high urinary nitrate concentrations were assumed to reflect high nitrate intake. Nitrite concentrations in gastric juice were also measured in high risk areas of Colombia [28]. In contrast, no nitrate or nitrite measurements were made in case-control studies in Japan [29] and of Japanese Hawaiians [30] in which gastric cancer risk was related to the consumption of certain foods, and to well-water use in Japan.

The principal features of these early studies in South America and Japan are summarized in the United States National Academy of Sciences review of the health effects of nitrate, nitrite and N-nitroso compounds [1]. Lacking reliable measures of nitrate intake in populations at differing risk, the evidence provided on the role of dietary nitrate in the etiology of gastric cancer is inconclusive. In the following discussion, attention has been focused on the results of more recent investigations on the relationship between gastric cancer and nitrate levels in drinking-water. Reports of studies in England [31-33], Chile [34], Hungary [35], Italy [36], Denmark [37] and France [38] have appeared in the last few years. Investigations in Colombia are continuing, and some preliminary observations are now available from China [39].

(a) Chile

After the demonstration of a strong statistical association between fertilizer use and gastric cancer mortality in Chile [22-25], a case-control study was carried out to test the association and to look for other etiological factors [40]. The study showed that gastric cancer was associated with a previous occupation in agriculture, and that patients had resided in high-risk areas during early life for longer periods than had controls. However, more detailed studies of nitrate intake revealed significantly higher nitrate levels in the urine of schoolchildren and in vegetables in a low-risk area [41]. Explanations are now being sought for these paradoxical findings which do not support the hypothesis that high nitrate ingestion is involved in the etiology of gastric cancer.

Zaldivar & Wetterstrand [34] examined the nitrate levels in drinking-water supplying 202 urban areas in 25 provinces in relation to gastric cancer death rates. The nitrate levels ranged from 0-30 ppm of nitrate-nitrogen. Only two provinces reported nitrate-nitrogen levels above 11.3 ppm. Zaldivar & Wetterstrand failed to find an association between nitrate

levels in water supplies and death rates from gastric cancer in either sex. When the 25 provinces were aggregated into six geographical areas, similar nonsignificant correlations were found (r = 0.1367 and 0.1143 in males and females, respectively). The authors suggest that the rural population, drinking-water mainly from natural springs and artesian wells, may be exposed to higher waterborne nitrate levels than urban dwellers but no data were available for analysis. The results presented provide no evidence that the high gastric cancer mortality in Chile is related to nitrate levels in drinking-water.

(b) Colombia

In Colombia, where the evidence for a link between gastric cancer and high nitrate ingestion is most persuasive, gastric lesions, such as superficial gastritis, chronic atrophic gastritis and intestinal metaplasia, have a high prevalence [42-44]. These recognized precursors of gastric cancer are associated with low gastric acidity and high gastric nitrite levels [28] and are most prevalent in impoverished communities, where gastric cancer is very common and the major cause of death. In gastroscopic studies among volunteers in a high-risk area, 75 % had some form of gastritis by 25 years of age [44]. A high corn diet was found to be associated with gastric lesions, but ingestion of lettuce, which contains vitamin C, was inversely related [43].
Wells with nitrate concentrations up to 68 mg/l are a feature of several high-risk areas in Colombia, and high urinary nitrate levels in single specimens [26] and 12-hour collections (Shabeen, personal communication) suggest that nitrate intake is also high. High urinary levels were not confined to drinkers of nitrate-rich well-water; high levels were also found in an area with nitrate-free water supplies, suggesting that locally grown food, rather than water, was the nitrate source.

(c) Denmark

Jensen [37] reported a higher incidence of gastric cancer in Aalborg, with an average nitrate level of 6.8 mg/l in its drinking-water, compared with Aarhus, with low-nitrate water supplies. Gastric cancer has decreased markedly in both towns over the 30-year period covered by the study, while nitrogen fertilizer use in Denmark has increased and the consumption of vitamin C, the best-known nitrosation inhibitor, has remained virtually constant. The cancer pattern seen in Aalborg is compatible with an assumption of a socioeconomic status lower

than that in Aarhus, but a comparison of the few available social class indicators revealed no obvious differences between the towns.

Urinary nitrate concentrations, assumed to reflect intake, were measured in two school classes in Aalborg and Aarhus. The distribution in Aalborg was skewed towards higher values, but no significant difference was found between the means (0.42 mmol/l and 0.28 mmol/l) or median values (0.33 mmol/l and 0.26 mmol/l) in the two towns, respectively. While Jensen suggested tentatively that his results support a possible weak role for nitrate in the etiology of stomach cancer, failure to demonstrate higher nitrate intake in Aalborg weakens this conclusion.

(d) England and Wales

Much publicity has been given recently to drinking-water as a source of nitrate, the increase in use of nitrogenous fertilizers over the last 30 years, and the rise of waterborne nitrate levels in the last 20 years. England and Wales, in common with many other countries, have experienced a marked decline in gastric cancer mortality. Death rates have fallen over the last 30 years at all ages in both sexes [45]. In fact, with the exception of the oldest age group, the decline in women began as early as 50 years ago, and in men about a decade later.

Age-specific incidence rates, available nationally for only the last 20 years, are also falling, except at ages 75+ [46]. In this oldest age group, an increase in 1974 probably reflects improved diagnosis of gastric cancer after the introduction of a simplified national scheme for cancer registration, rather than a genuine increase in incidence. The prognosis for patients with gastric cancer remains poor: only 7% of registered cases survive 5 years. The substantial decline in mortality is not therefore due to an improvement in survival.

The decrease in gastric cancer mortality is apparent in all regions of England and Wales, with no apparent association between the rate of decline and the rate of increase in use of nitrogenous fertilizers [32]. Furthermore, a clear inverse relationship occurs between cumulative fertilizer usage (from 1938-72) and gastric cancer mortality (in 1969-73) in the rural aggregates of the standard regions, the traditionally agricultural areas in the south and east of England with higher cumulative fertilizer usage experiencing lower mortality [32].

Bearing in mind that the movement of nitrate from land surface to underground water sources is slow and that a time interval occurs between exposure and death, the full impact of the intensive agricultural activity of recent years may still

need time to be manifest. However, the trends in health statistics give no cause for concern at present and provide no suggestion that increasing use of nitrogenous fertilizers plays any role in the etiology of gastric cancer in England and Wales.

One of the earliest studies of the relationship between nitrate levels in drinking-water and gastric cancer was carried out by Hill et al. [27] in 1973 in Worksop, a Nottinghamshire mining town where the public water supply had long contained an average nitrate level of 20.3 mg/l - well above the WHO guideline value of 10 mg/l - but still a level regarded acceptable for drinking purposes. Hill et al. suggested that by comparison with national rates, mortality from gastric cancer in women in Worksop from 1963-71 was some 60% higher; it was also higher than mortality in nine neighbouring towns supplied with low-nitrate water. One of these (Chesterfield) also had a mortality level significantly above the national average. On the other hand, male mortality in Worksop was similar to several other towns and not significantly increased.

In 1980, Davies [31] made a more detailed study of Worksop and other mining and nonmining towns in Nottinghamshire. She had access to revised population estimates and data over a longer period of time, and she also adjusted for differences in social class distribution and proportion of miners when calculating standardized mortality ratios (SMR), as members of the lower social classes and miners are known to have higher mortality from gastric cancer anyway. The female SMR for Worksop decreased in significance with each adjustment until finally, although still raised at 131, it did not differ significantly from the national average. Similar results were obtained when deaths over a longer period of time, from 1958-75, were examined. Davies concluded that, if allowance were made for differences in the social class structure and the number of miners in each town, Worksop showed little indication of having a higher death rate from gastric cancer in either sex than neighbouring mining towns with low-nitrate water.

Correlation studies by Fraser & Chilvers [32] of gastric cancer mortality during 1969-78 in 32 rural districts in eastern England in relation to nitrate concentrations in the public water supplies since about 1955 showed no consistent pattern. In the Anglian Water Authority area, they demonstrated a significant trend in male gastric cancer mortality with increasing concentrations of waterborne nitrate, but the trend diminished in strength over time and was not apparent in females. While domestic water supplies seem an unlikely explanation for these findings, the trend in males could neither be explained in terms of differences in social class distribution nor in the proportion of miners, chemical workers

or agricultural workers in each nitrate category. Male
mortality in 12 rural districts in the Yorkshire Water Authority
area followed a similar pattern, but the difference in gastric
cancer mortality between the nitrate categories was not
significant and the findings in Yorkshire females were
inconsistent over time.

While intensive agricultural activity is the major factor
responsible for rising nitrate levels in underground water
sources, increased recycling of sewage effluent is a
contributory factor in lowland rivers. Beresford [33] studied
the relationship between re-use of water and hazards to health
in the London area, using nitrate as one of several indicators
to assess the degree of re-use. Mortality from different
causes, principally cancer, was examined for 29 London boroughs
from 1968-74. Socioeconomic characteristics of the boroughs and
variations in their size were found to account for any
statistical associations between water re-use and each cause
studied. In a recent more extensive study of 253 towns in the
United Kingdom, Beresford (personal communication) found no
evidence of an association between nitrate levels in
drinking-water and mortality from cancer in general or gastric
cancer in particular.

(e) France

An epidemiological study in 753 communes in northern France
[38] found no association between drinking-water nitrate levels
from 1974-76 and digestive or urinary cancer mortality rates
from 1968-75. Mean nitrate concentrations were less than
21.4 mg/l, with 93% under 9.7 mg/l.

(f) Hungary

In Hungary, another country with high mortality from
gastric cancer, shallow wells often contain high nitrate
levels. An epidemiological study was started in 1975 to examine
the relationship between drinking-water nitrate, methaemo-
globinaemia, soil type and gastric cancer in 230 localities in
the county of Szabolcs-Szatmar [35]. Each locality was
allocated to one of four groups according to gastric cancer
incidence (≤ 20 per 100 000 or ≥ 20) and well-water nitrate
concentration (< 22.6 mg/l or > 22.6).

A high incidence of gastric cancer was found in 60% of all
localities, and the majority of these (127 out of 139) had high
concentrations of nitrate in their drinking-water; of
91 localities with a low incidence, 78 also had high-nitrate
water. The 13 localities where both gastric cancer incidence
and nitrate levels were low and the 127 localities where both

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were high would be consistent with a role for nitrate in the etiology of gastric cancer. However, such consistency is not shown in 12 localities where nitrate levels were low but gastric cancer incidence high or in 78 localities with low gastric cancer incidence yet high nitrate levels in drinking-water. The small size of many of the localities and the absence of information on the variability of well-water nitrate levels cast doubt on the reliability of both the incidence rates and the waterborne nitrate levels in this study.

(g) Iran

Nitrosamines, nitrates and nitrites were not incriminated in the detailed studies of oesophageal cancer in northeast Iran [47].

(h) Italy

The publication by Amadori et al. [36] of some preliminary data on a group of 92 Italian farmworkers received wide publicity when well-water nitrate levels of 10 mg/l were claimed to be causing gastric cancer in these workers. This conclusion cannot be inferred from the data presented in the paper, for no information is given on the levels of nitrate in the drinking-water of the urban population at lower risk with whom the farmworkers were being compared. Furthermore, the farmworkers were heavily exposed to agricultural chemicals, including carbamates which can react with nitrites in the soil and crops to form N-nitroso compounds.

(i) People's Republic of China

Preliminary data from China also show that levels of nitrate and nitrite in vegetables and drinking-water are higher in areas at high risk of gastric cancer than in low-risk areas [39]. Nitrate and nitrite levels in fasting saliva and gastric juice of patients with chronic gastritis were also found to be higher in high-risk than in low-risk areas, with the incidence of chronic gastritis running parallel to gastric cancer mortality rates.

Chinese scientists have suggested that nitrosamines are likely to be causative agents for oesophageal cancer in China [48]. A diet rich in nitrates, nitrites, secondary amines and nitrosamines but low in vitamin C is a feature of high-risk areas, where oesophageal dysplasia is also prevalent. A great deal of information on oesophageal cancer and possible risk

factors has been gathered in China, but while nitrosamines are among the prime suspects, they have not been established as the causative agents.

7.2.2 Clinical studies of carcinogenicity

Several investigators have demonstrated an association between high gastric nitrite levels, low acidity and presence of N-nitroso compounds [49]. In 301 gastric juice samples from 267 untreated subjects, including 50 healthy volunteers, nitrosamine concentrations rose progressively with pH, and patients with conditions associated with hypochlorhydria showed correspondingly high mean levels. Thus, patients with chronic gastritis, gastric ulcer, a partial gastrectomy, pernicious anaemia and gastric cancer, where the pH was greater than 3, had higher nitrosamine levels than normal subjects and patients with conditions associated with more acidic stomach contents.

An increased risk of gastric cancer in conditions associated with low gastric acidity is well recognized and lends support to the hypothesis that N-nitroso compounds may be involved in its development. Their formation can be readily inhibited in vitro and in vivo by antioxidants such as vitamin C, and Reed et al. [50] have now demonstrated for the first time in humans a significant lowering of gastric juice N-nitroso compounds by ascorbate treatment in 51 achlorhydric subjects. Clearly, this observation may have important implications for preventing gastric cancer in high-risk subjects if N-nitroso compounds are shown to be causative agents.

7.2.3 Conclusions about gastric cancer

No convincing evidence of a relationship between gastric cancer and consumption of drinking-water containing nitrate levels up to 10 mg/l has emerged. Furthermore, no firm epidemiological evidence has been found linking gastric cancer and drinking-water containing higher levels of nitrate, but a link cannot be ruled out due to the inadequacy of the data available. Gastric cancer is declining in most countries, and the risk from nitrate, if any, would appear restricted to individuals with conditions associated with low gastric acidity, rather than to the population in general. Very few studies have considered human cancers other than gastric cancer in relation to nitrates, and none of them provides convincing evidence that nitrate ingestion influences cancer incidence at other sites.

7.3 Other effects

7.3.1 Birth defects

Scragg et al. [51] reported a descriptive study indicating a localized excess of congenital malformations from 1968-76 in Mount Gambier, South Australia. The malformations mainly responsible for the increased rate were neural tube defects and malformations affecting multiple systems, birth defects which experimental evidence links with teratogenicity of N-nitroso compounds.

A subsequent case-control study [51] demonstrated an association between the occurrence of congenital malformations and the estimated nitrate concentration of water consumed during pregnancy. Compared to water with less than 1.1 mg/l of NO_3-N, water nitrate concentrations of 1.1-3.3 mg/l were associated with a doubling of risk, while those above 3.3 mg/l were associated with a three-fold increase. However, further studies of the consumption of nitrates in food and water in South Australia [52] have shown that the association is not likely to be causal, and Scragg et al. have suggested that unidentified teratogenic factors, which are correlated with water source and hence with nitrate levels in water, may be responsible. A descriptive study in eastern England has provided no evidence that congenital malformations are associated with nitrate levels in water supplies (Fraser, in preparation 1984).

7.3.2 Cardiovascular effects

Although glyceryl trinitrate is used with benefit in the treatment of angina pectoris, some workers have suggested that chronic exposure to high levels of nitrate in drinking-water may have adverse effects on the cardiovascular system. A statewide study of municipal water supplies in Colorado in 1960 suggested that the significantly higher hypertension risk in the eastern plains might be associated with higher nitrate concentration [53]. This finding was further investigated by Malberg et al. [54] who reported an earlier onset of hypertension among residents of communities exposed to nitrate levels of 4.3-28.2 mg/l compared with communities that had nitrate-free drinking-water. On the other hand, a British regional heart study has demonstrated an inverse relationship between cardiovascular mortality and nitrate concentration in water supplies [55].

7.3.3 Effects on the thyroid

Studies in the German Democratic Republic on the incidence of goitre in children and adolescents in relation to the iodine supply have suggested that goitrogenic noxious agents may also be involved in the disease's etiology [56-58]. Attention has been focused on the effect of nitrates in drinking-water on the thyroid gland [59,60]. In 1955, Hettche [61] stated that nitrates might be involved in the etiology of endemic goitre in the Netherlands. This opinion was supported by investigations by Wurmbach et al. [62] and Körber [59] who investigated livestock in regions where goitre is endemic. The competitive mechanism leading to the inhibition of iodine uptake by nitrate ions has been known for a long time [63]. It also plays a role in the process of iodine resorption in the gastrointestinal tract [64]. However, little information is available on the dose-effect relationship and possible synergistic effects of nitrate and other goitrogenic noxious agents.

For this reason, studies have been performed on rats by Höring et al. [65] to investigate the influence of nitrate on thyroid function when administered alone or with goitrogenic substances.

Thyroid function was tested by determining ^{131}I uptake in the thyroid gland, ^{131}I serum level, ^{131}I incorporation into protein-bound iodine within 24 hours, thyroid gland mass, epithelial height, and the histological pattern index. These data provide information on the iodine uptake capacity of the thyroid gland, the extent to which the extra-thyroidal iodine pool is involved - and thus indirectly on the capacity of the intra-thyroidal iodine pool - as well as on possible disturbances in the hormone synthesis.

In a further study, the relative amounts of mono- and diiodotyrosine, triiodothyronine, thyroxine and inorganic iodine in thyroid homogenates were determined as described by Knopp and co-workers [66]. After confirming the correlation between thyroid function and the histometric evidence of morphological changes in studies by Zimmermann & Emrich [67] in rats, and by Sidor & Kovac [68] in pigs, additional histological examinations of the thyroid gland were performed. The epithelial height proved to be a useful parameter. In addition, the size and shape of follicles were empirically assessed and formed the bases for allocating a histological pattern index to each microscopic slide [69].

In the study by Zimmermann & Emrich [67], rats were gradually administered nitrate in drinking-water - at doses of 9.1, 45.2, 270 and 900 mg/l of NO_3-N in the course of 100 days. As indicated in Figure 1, already at a nitrate dose of 9.0 mg/l, a significantly higher epithelium and an increased

histological pattern index were found within 13 weeks. In contrast, the thyroid gland mass and the ^{131}I uptake were changed only slightly.

In the study by Sidor & Kovac [68], 270 mg/l of NO_3-N per litre was administered in the drinking-water during 1, 2, 4, 6 and 12 weeks. The results (Figure 2) indicated: (a) no changes in thyroid gland mass; (b) a decrease in ^{131}I accumulation in the thyroid gland from the 6th week onwards; (c) a significant increase in ^{131}I serum level from the 4th week onwards; (d) a slow increase in epithelial height which becomes significant only during the 12th week; and (e) an increased histological pattern index during nitrate exposure.

In summary, the above shows that even at a dose of 9.0 mg/l of NO_3-N in drinking-water and under conditions of normal iodine supply, nitrate causes histomorphological changes in rats within a short period of time. These changes result from a continuous compensation process and can be considered the beginning of goitre development. Under the influence of the nitrate ion, the intrathyroid iodine pool is reduced, making the gland more sensitive to other goitrogenic noxious agents. Goitrogenic agents with a different mechanism of action also produce a reduction of the intrathyroid iodine pool.

7.3.4 Conclusions concerning other effects

Studies relating congenital malformations and cardiovascular effects to nitrate levels in drinking-water have not produced consistent results. Some animal studies indicate that chronic exposure to high levels of nitrates can reduce the intrathyroid iodine pool and thus render the gland more sensitive to goitrogens. However, whether or not exposure to nitrate is an etiological factor in human goitre remains to be determined.

Fig. 1. Effect of nitrate administration on four thyroid parameters in the rat (Data from [65,69])

The difference in percent of mean value for control group is shown. For the index, the difference multiplied by 10 is shown. ▨ ^{131}I uptake in thyroid gland; ▥ thyroid gland mass; ☐ epithelial cell height; ☰ index of morphological pattern; X = value signif. dif. from control; O = value signif. dif. from group 2; Δ = value signif. dif. from group 3; p ≤ 0.05
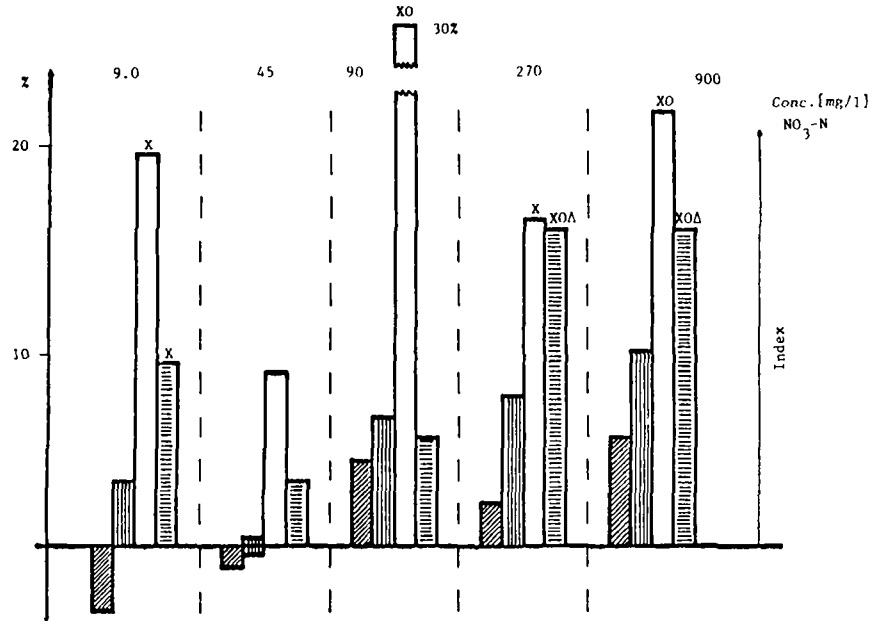
Fig. 2. Time-dependent changes of five thyroid parameters during continuous administration of 270 mg/1 of NO_3-N (Data from [65, 69])

The difference in percent of the mean value for the control group is given.

▦ Thyroid gland mass; ▨ ^{131}I uptake by thyroid; ▦ ^{131}I serum level; ☐ epithelial cell height; ▤ index of histological pattern; X = value signif. different from control, $p \leq 0.05$; XX = value different from control, $p \leq 0.06$
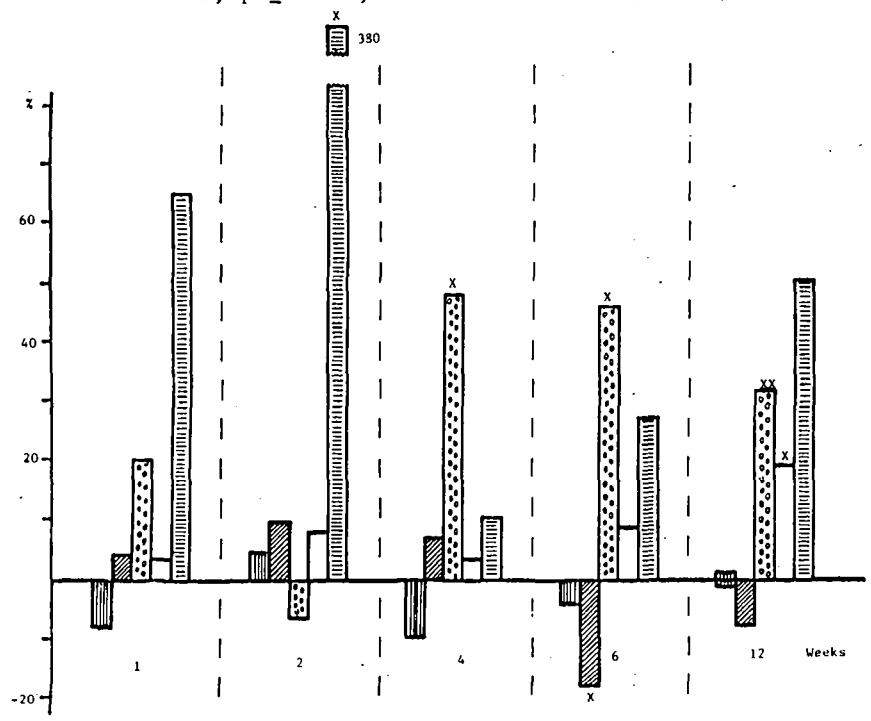
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8. TREATMENT TECHNOLOGIES FOR REDUCING NITRATE LEVELS IN DRINKING-WATER

8.1 Hydrogeological investigations

Hydrogeological investigations should be carried out prior to any treatment decision. They may shed some light on the origin of nitrates and lead to relatively simple control measures when the pollution source is localized.

8.1.1 Isotopic tracing

The use of synthetic nitrogenous fertilizers in industrialized countries, which has increased markedly since 1950, has been blamed for the rise in nitrate levels in drinking-water sources there. However, cases exist where the appearance of nitrate in groundwaters is due to more local pollution (e.g. wastewater plants, cattle feed-lots), and direct analysis of the water composition can now identify the origin of the nitrogenous compounds.

Natural isotopic ^{15}N has been successfully measured in the water from aquifers in the Paris region [1,2]. The amount of ^{15}N as a function of total nitrate shows the origin of NO_3. These results are summarized in Figure 3. The availability of other chemical markers (phosphorus and boron from detergents, potassium and ^{32}S from fertilizers, and the natural tracers ^{3}H, ^{17}O and ^{14}C), considered in conjunction with the nitrate analysis, provides some additional information on the origin and rate of change of the nitrates.

These techniques have already made possible the distinction between pollution by nitrates due primarily to fertilizers and cases of more complex pollution involving an input of domestic effluent. This type of study also permits nitrates to be dated. If they are old in origin, a reduction in the input of fertilizers will not permit the problems to be solved in the short term. If they are more recent, then changes in agricultural practice can, in a few cases, have a very rapid effect on resource quality. However, reduction of the nitrate level by minimizing the input of fertilizers often depends on local economic and political considerations and its implementation is neither easy nor quick.

8.1.2 New resources for dilution or replacement

Hydrogeological investigations will also provide information on the availability of better water resources which

could be used for dilution or more simply for replacement of polluted ones. The dilution technique seems, of course, the easiest way to solve the problem. However, the amount of low-nitrate water necessary to reduce the nitrate level by mixing with a polluted resource becomes rapidly preponderant. Figure 4 summarizes this process, showing the proportion of low-nitrate water versus the polluted one needed to reach a final nitrate concentration of 6.8 mg/1.

8.1.3 Treatment in the ground

Ground investigations will provide information on the characteristics of the polluted aquifer and indicate whether or not direct treatment in the ground is feasible. This technique, which is still being studied and is not yet operational, involves transforming the nitrates in the aquifer into gaseous nitrogen. This change is achieved by injecting a suitable reducing agent through wells or boreholes. In principle, the reaction could go from nitrate to nitrite and then to nitrogen and eventually to ammonia. The problem is thus to control the reaction to produce maximum nitrogen which can evolve from the water. Otherwise, either nitrite or ammonia will have to be retransformed to nitrate when water is abstracted before its distribution. Various reagents have been applied, but this technique, which could result in aquifer blocking, is far from being a well-validated practical proposal.

Groundwater recharge and bank filtration have also been suggested as ways to reduce nitrates. They need further study, but as a general rule, the same precautions as stated above should be taken.

8.2 Removal of nitrates

Two broad categories of treatment can be distinguished: physicochemical and biological.

8.2.1 Physicochemical processes

(a) Reverse osmosis

Reverse osmosis refers to a separation technique making use of selective membranes. By applying a pressure greater than the osmotic pressure, the solvent is caused to migrate through the membrane, which therefore retains the dissolved species. This technique thus makes possible the extraction of a pure solvent from a concentrated solution under a pressure gradient.

Fig. 3. Example of distribution of ^{15}N in groundwaters (a) and their corresponding origin (b) (From [7])

● Calcium layer under agricultural land; ○ layer under forested areas; ∇ calcium layer from Champigny to Brie-Comte-Robert
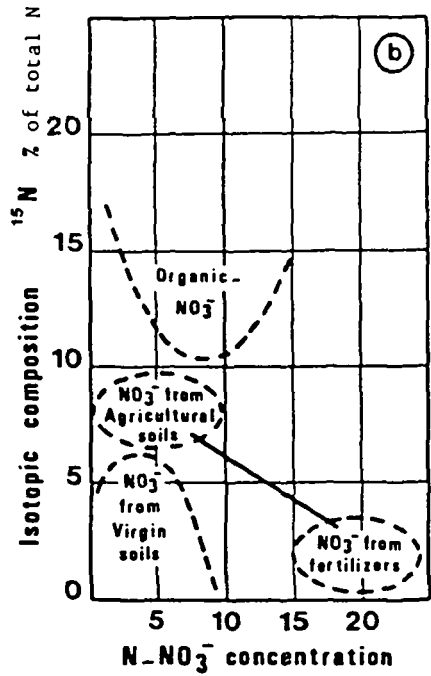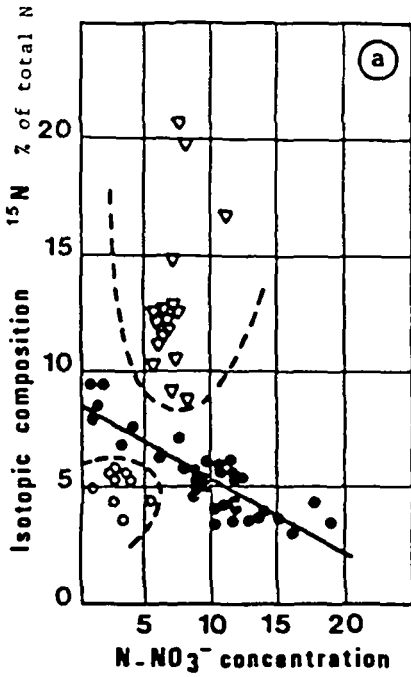
Fig. 4. <u>Calculated rate of dilution versus nitrate concentration</u>
<u>in contaminated and noncontaminated resources to reach a</u>
<u>concentration after mixing 6.8 mg/1 of NO_3-N</u>
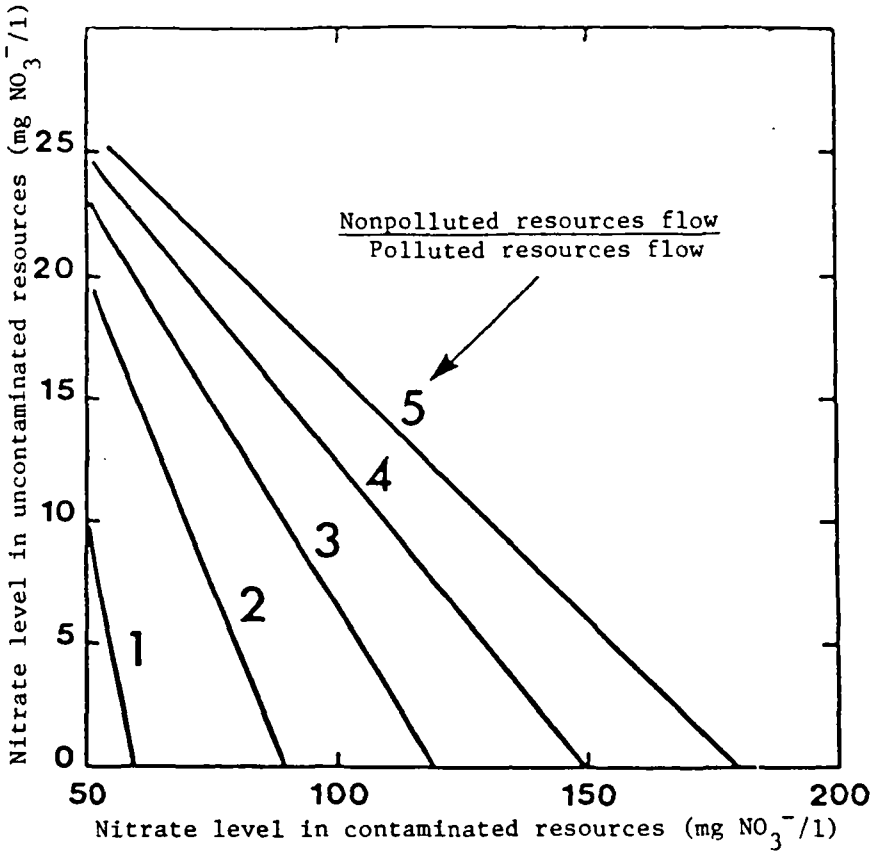
A schematic drawing of an installation of this type is shown in Figure 5. The membranes in current use are essentially composed of the following materials: cellulose acetate, cellulose triacetate, aromatic polyamide, and poly(ether/amides). These materials are employed in a modular form, thus providing a maximum surface area per unit volume. The modules, which are commercially available, can be divided into the following categories:

(1) flat modules (progressively removed and replaced);
(2) tubular modules with an installed surface area of 200 m^2/m^3 of module;
(3) spiral modules with an installed surface area of 1000 m^2/m^3; and
(4) hollow fibre modules with an installed surface area of 15 000 m^2/m^3.

At present, use is made predominately of types (1) and (2). The efficiency obtained depends on the pressure applied, the solute concentration, and the yield of purified water. As an example, the following efficiencies can be given for the removal of nitrates: 85% removal at 30 bars; 95% removal at 60 bars. Figure 6 shows an example of the results obtained.

The limitations of reverse osmosis can be summarized as follows: (i) retention of the species is nonspecific: this type of treatment alters the initial composition of the water considerably; (ii) pretreatment is an absolute requirement in order to avoid fouling the membranes; and (iii) concentrated wastes which require removal and sometimes treatment are present. These limitations explain why this process is not yet employed for nitrate removal, despite its development in desalination. The reliability of reverse osmosis, however, makes it a possible technique when "point-of-use" treatment should be applied (see below).

(b) **Ion exchange**

This technique has been the subject of several studies [3,4] as well as industrial applications. On Long Island, New York, a plant operating since 1974 reduced a nitrate level of 20.3-29.4 mg/l to approximately 0.45 mg/l; its nominal throughput is 270 m^3/h. In Great Britain, two plants which have been constructed by the Anglian Water Authority have throughputs of 100 and 200 m^3, respectively.

The principal questions raised by the use of such a technique relate to the appropriate choice of resin according to the potential risks of a chemical release from the resin itself,
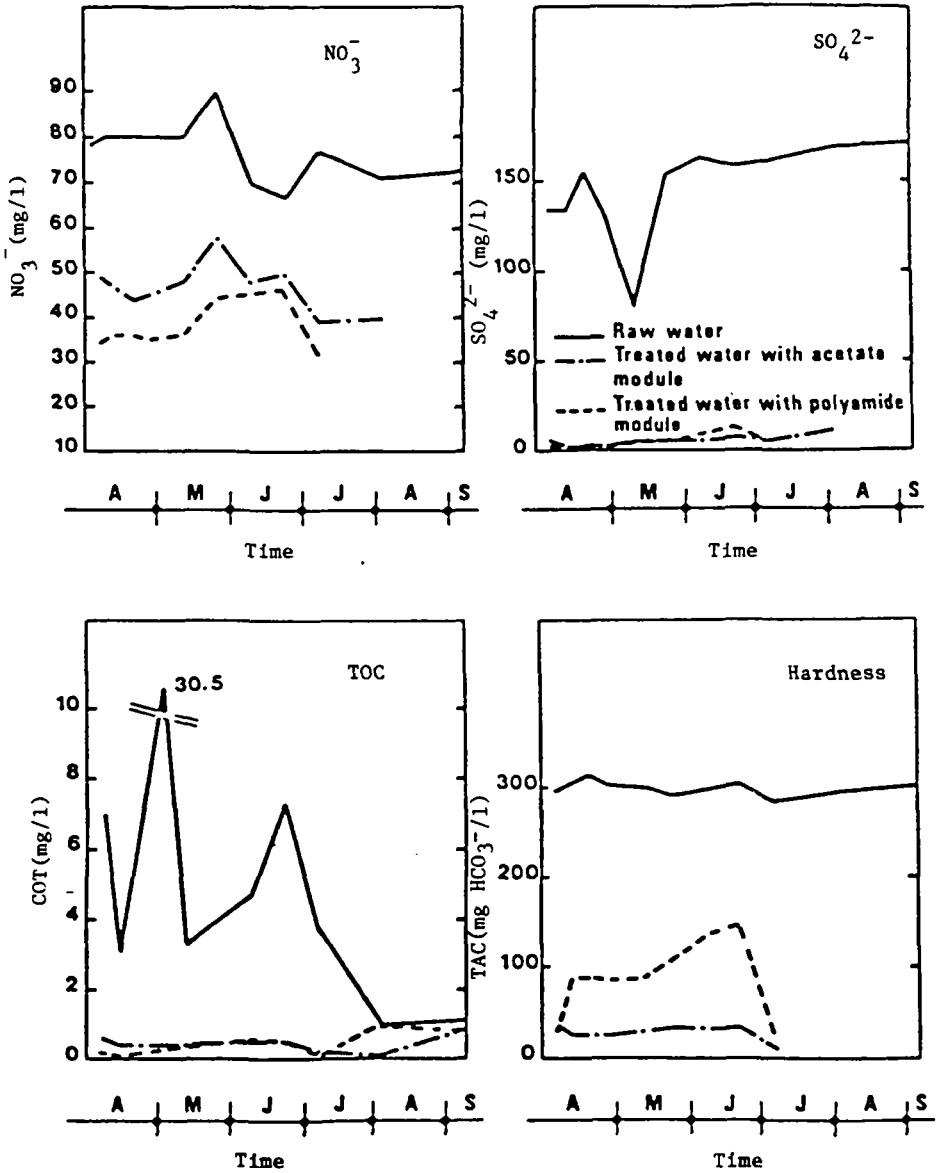
Fig. 5.   Schematic representation of a reverse osmosis process
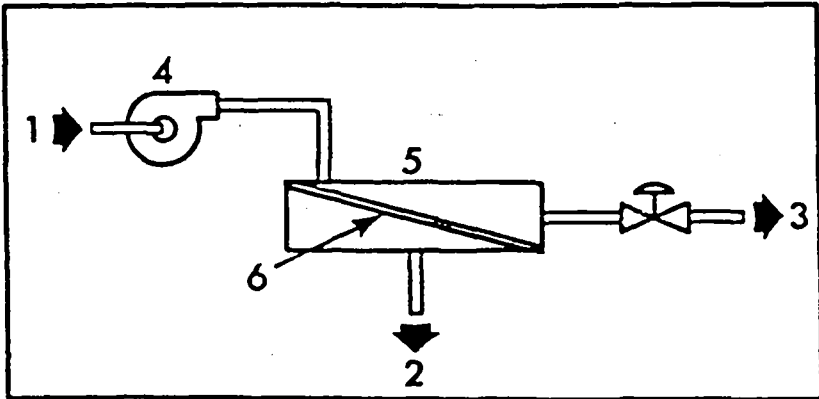
1_ Raw water   4_ High pressure pump
2_ Treated water   5_ Reserve osmosis module
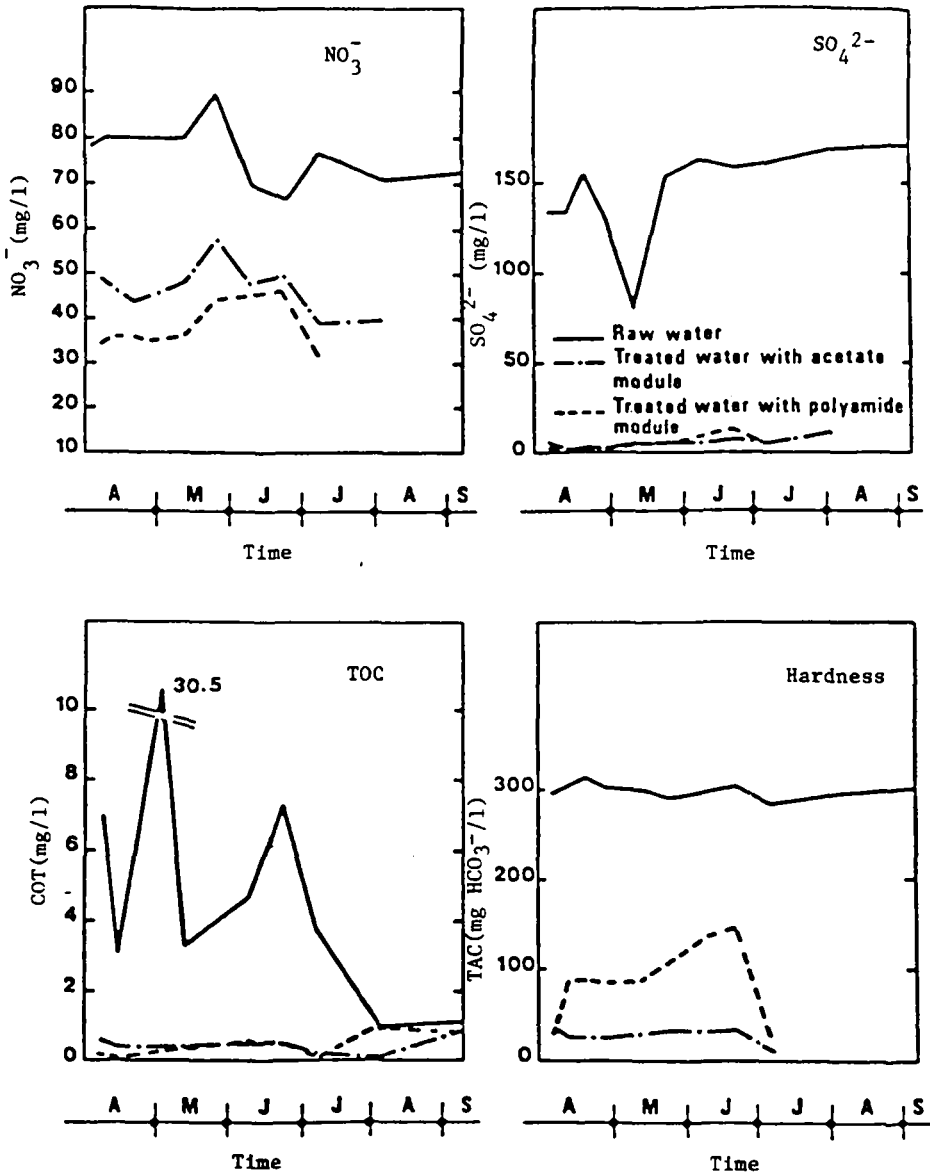3_ Concentrate   6_ Semi-permeable membrane

Fig. 6. Example of the efficiency of reverse osmosis units

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Fig. 6. <u>Example of the efficiency of reverse osmosis units</u>

and the operating conditions, which must be adapted to the composition of the water being treated.

Choice of resin: For taste, odour and toxicity reasons, the choice is limited to the strongly basic Type I or Type II resins. Type I resins are chemically more stable, while Type II are relatively sensitive to the Hofmann degradation, particularly in waters containing dissolved oxygen. On the other hand, Type II resins have a higher capacity and can be regenerated more efficiently than Type I resins. The final choice will depend on the release of cyclic and amine compounds after a resin-conditioning stage. Several anion exchangers for which the release of anions, styrene and divinylbenzene is below the limit of detection by analytical methods have been identified at the present time (principally Type I exchangers).

With regard to this type of resin, the various ions are fixed in the following order of affinity:

$$SO_4^{2-} > NO_3^- > Cl^- > HCO_3^- > OH^- > F^-$$
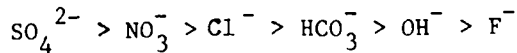
The nitrates will not be the only ions fixed by a resin which are initially in the form of chloride, and the capacity of the resin for the sum of nitrate + sulfate and possibly carbonates in the water to be treated (Fig. 7) must be considered.

Fig. 7. Breakthrough curves for ion exchange treatment (From [4])

Volume of treated water per volume of resin
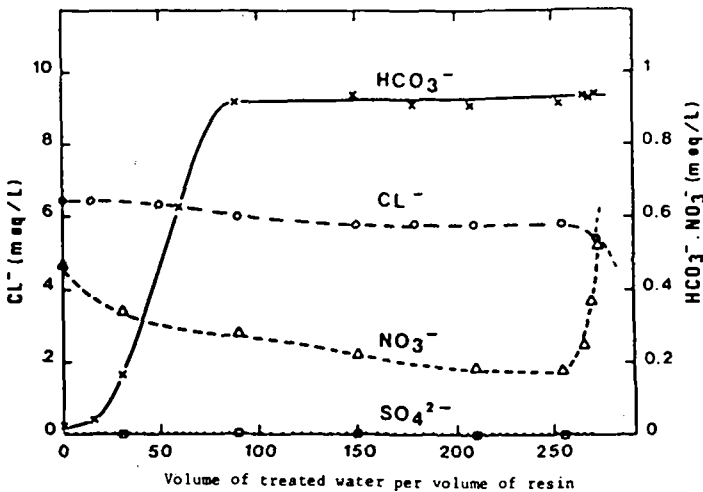
Operating conditions: These will vary in each particular case. Conventionally, the following operating conditions are used for a strongly anionic Type I resin:

- volume load: 15-35 m^3 of water/m^3 of resin per hour;
- resin bed depth: approximately 1-1.5 m;
- exchange capacity: approximately 0.8-1.2 eq ($NO_3 + SO_4$)/1 of resin; and
- countercurrent regeneration with a sodium chloride brine, with a concentration of 5-10%, with recycling of the last 40% of each regeneration with the following one, which reduces the consumption of the regenerating NaCl.

In conclusion, there is now considerable experience of nitrate removal by ion exchange. It appears to be simple in use and with its reliance only on physicochemical processes, it offers excellent reproducibility. However, the nitrates are concentrated in the regeneration brine. Therefore, provision must be made for their disposal in an authorized way, which can occasionally present considerable problems. Finally, substantial changes are made to the ionic balance of the water, which could lead to increased corrosivity.

(c) Electrodialysis

This technique, which employs the principle of electrolysis combined with the use of selective membranes, can be applied to the removal of nitrates from drinking-water. For this purpose, membranes must be designed with high performance, low resistance and increased selectivity. Using membranes produced by the application of a 2-10 micron layer of 4-benzyl pyridinium CA to a porous substrate, and an applied current of 1 mA/cm^2, the following results can be obtained:

| Compounds	Removal efficiency %
NO_3^-	40-60
Cl^-	12-22
HCO_3^-	3-18

The principal characteristics of this treatment are average specificity and limited efficiencies, need for a pretreatment, and presence of a fairly concentrated waste. On the other hand, this technique does not require regeneration and could be applied in certain cases. With the introduction of new, more specific membranes, it could be greatly developed.

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8.2.2 Biological treatment

Although long used in slow filtration and activated sludge processes, bacterial microorganisms capable of reducing nitrates have been employed only recently for specific treatment applications. Biological denitrification as applied to drinking-water consists of fixing the bacteria on a substrate to increase the concentration of active microorganisms, while keeping them inside the biological reactor, which makes possible an increase in the efficiency of the biological reaction. These processes use autotrophic or heterotrophic bacteria which convert nitrates into gaseous nitrogen, provided they have an oxidizable substrate which may be a solid such as sulfur, a liquid such as a carbon compound, or a gas such as hydrogen.

(a) Denitrification using hydrogen

This process [5], of which little is known so far, employs autotrophic bacteria to carry out the reaction:

$$2NO_3^- + 5H_2 \leftrightarrows N_2 + 4H_2O + 2OH^-$$
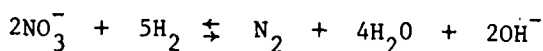
To make use of this process, hydrogen must be introduced into the reactor itself or dissolved beforehand in the water to be treated.
Using a column of activated carbon with a bed depth of 1.60 m, the results shown in Table 9 were obtained:

Table 9. Examples of denitrification efficiency using hydrogen (From [5])

|	1	2	3
Velocity (m/h)	6	3	1.2
Contact time (minutes)	16	32	84
Quantity of NO_3^- removed (mg/l)	65	80	80
Quantity of NO_2^- present (mg/l)	18	15	0
Initial pH	7.6	7.5	7.4
Final pH	8.5	9.0	9.0
Dissolved O_2 (mg/l)	7.5	7.5	7.5

From these results, the following observations can be made. As might be expected from the chemical reaction, the pH rises during the removal of nitrates. In addition, nitrites appear at velocities likely to be used in an industrial plant. The biological reaction is incomplete and operating at lower velocities (1.2 m/h) is necessary to convert completely the nitrites into gaseous nitrogen. By carrying out a recycling with a predissolution of hydrogen, the nitrites can be removed in only 60 minutes [5], which corresponds to a velocity of 1.6 m/h. These results are shown in Figure 8.

When applied industrially, this process will give rise to two problems: a long contact time required to avoid nitrites and use of dangerous gas such as hydrogen, which involves explosive risks.

(b) Denitrification on a sulfur substrate

This process [6,7], which employs the bacteria Thiobacillus denitrificans, has been known for many years. The reagent employed is solid and must be added periodically to the substrate material. Under anaerobic conditions, the autotrophic bacteria utilize the energy released by the reaction (in which $C_5H_7NO_2$ represents the bacterial cell):

$$11S + 10NO_3^- + 4CO_2 + 7.6H_2 + 0.8NH_4^+ \rightarrow 0.8C_5H_7NO_2 +$$
$$11SO_4^{2-} + 12.8H^+$$
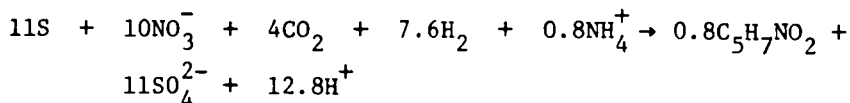
These specific bacteria are fixed in various proportions on a sulfur substrate or on a substrate of sulfur and marl when use of carbonates is also desired.

Recent experimental results [6] are shown in Table 10 and Figure 9. These preliminary results show that flow velocities obtained are low and efficiency drops appreciably beyond 2 m/h. However, the use of a mixture of sulfur and carbonates increases the performance appreciably. In addition, the reaction is accompanied by the formation of sulfates - 1.7 milligram of sulfate per milligram of nitrate. Finally, the sulfur medium must be regenerated periodically, which can present practical problems, particularly in the mixing of the substrate.

Additional studies may improve the efficiency of this process and lead to the development of methods with shorter contact times. This direction would definitely render this technique even more attractive.

Fig. 8. Examples of denitrification performance of a biological process using hydrogen (From [5])
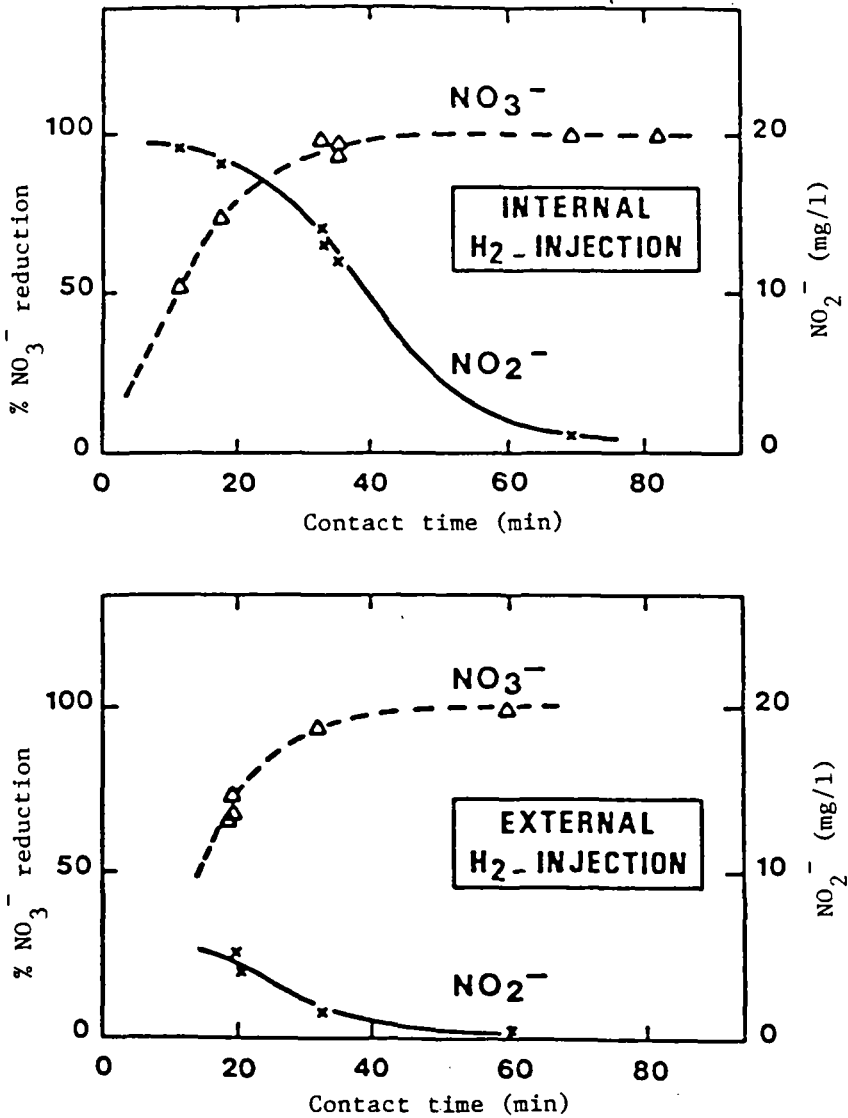
Table 10. <u>Sulfur denitrification: efficiency (%) versus flow rate and filter medium composition</u>

Substance	Flow rate ($m^3/m^2/h$)			
	0.5	1	1.5	2
Sulfur 100%	90	65	35	25
Sulfur 50%)				
+)	95	85	70	60
Carbonates 50%)				

(c) <u>Heterotrophic denitrification</u>

To date, this treatment process [7-10] seems the most attractive for industrial treatment of water. It uses heterotrophic microorganisms which require a carbon-containing nutrient. The process can be represented schematically by the following bacterial reactions:
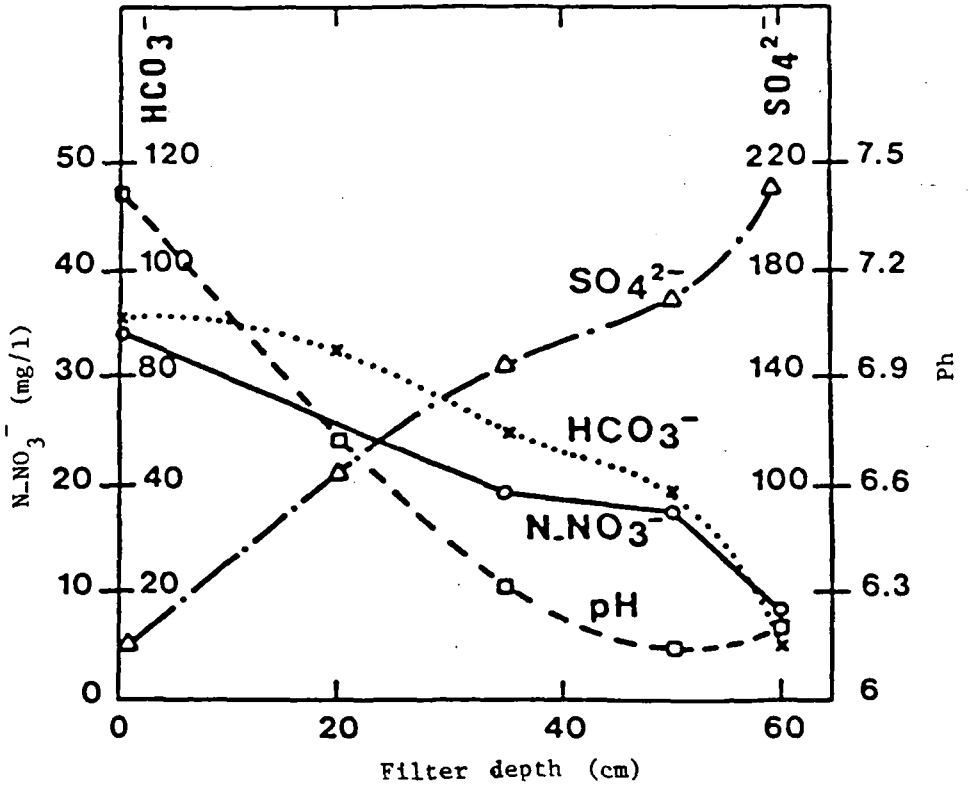With acetic acid as a carbon substrate:

(I) $8NO_3^- + 5CH_3COOH \longrightarrow 4N_2 + 10CO_2 + 6H_2O + 8OH^-$
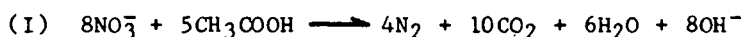
With ethanol as a carbon substrate:

(II) $12NO_3^- + 5C_2H_5OH \longrightarrow 6N_2 + 10CO_2 + 9H_2^- + 12OH^-$

The release of OH^- is in principle compensated by the formation of carbonic acid, and the overall change in pH remains small.
Groundwater sources do not generally contain the quantity of organic carbon required to establish reactions (I) or (II). Therefore, a biodegradable carbon compound which complies with drinking-water quality regulations is necessary. Compounds such as glucose, sugars and the like, as well as nutrients of the methanol type, have been rejected. For now, only ethanol and acetic acid have been selected. Using these substrates, utilization efficiencies of approximately 80-85% are obtained, which indicates the importance of the nutrients remaining in the treated water. However, the advantages and disadvantages of these various carbon sources in terms of treatment and cost effectiveness, as well as in the light of residuals and byproducts in the treated water, should be thoroughly examined.

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Fig. 9. Variation of water composition during biological denitri-
fication with sulfur and carbonates

Denitrification of drinking-water is carried out in a reactor with cells attached on a medium either in a fixed or an expanded bed with a downward or an upward water flow. Each technical difference presents advantages and disadvantages. The characteristics of the bed medium are important. The surface of the granule should provide for easy bacterial attachment and the particle size (usually from 1.5 mm up to 5 mm) should be a good compromise between low head loss, good expansion (when the bed is expanded) and high biomass.

The process can be divided into four stages, each with a different function (Table 11 and Figure 10).

The bacteria of the heterotrophic processes seem to respond well to the requirements of a plant-scale application (e.g. interruption of carbon supply, total plant stoppage, changes in flow-rate) and, with at times some delay in the response, enable 100% nitrate removal.

While no discharge of a nitrate concentrate occurs, the heterotrophic processes produce a sludge which must be removed. For example, the removal of 60 kilograms of nitrate per day (which represents roughly the capacity of a 100 m^3/h plant) produces a quantity of sludge corresponding to that from a population equivalent to 100 inhabitants.

In conclusion, these heterotrophic biological processes appear reliable and at present the majority of the industrial plants for nitrate removal use this technique. Three full-scale plants are in operation in France, two or three in England and several others in the Netherlands and Federal Republic of Germany. Numerous pilot plants are also being tested.

Table 11. Function of the different stages in a denitrification plant

| | Stage | | | |
	Biological	Aeration	Filtration	Disinfection
NO_3^-	++	0	+	0
TOC	-	0	+	0
Dissolved oxygen	-	++	0	0
Turbidity	-	0	++	0
Bacteria	-	0	+	++

++ = Large positive effect 0 = No effect
 + = Positive effect − = Negative effect

Fig. 10. <u>The heterotrophic denitrification process</u>
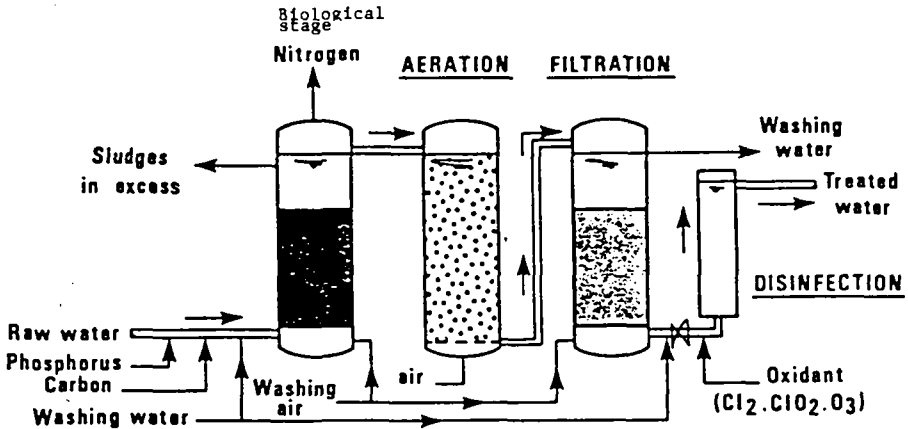
8.3 Economic study

The following discussion will focus on these treatments: dilution of the nitrates, treatment by reverse osmosis or ion exchange, and removal of the nitrates by denitrification with sulfur, hydrogen or a carbon substrate.

All the figures given here can only indicate the order of magnitude. The real cost of treatment will, of course, depend upon local conditions, together with the individual characteristics of the water to be treated and the cost, if any, for effluent disposal. All the figures given below are expressed in French francs (January 1984, 1 FF = 0.12 US$ = 0.85 DKr; F. Fiessinger, personal communication).

8.3.1 Capital costs

The data in Figure 11 clearly show that ion exchange treatment is undoubtedly the least costly, followed by heterotrophic biological denitrification. Because the operating velocities are very low (of the order of 1 m/h), the investment costs of autotrophic denitrification appear to be very high and of the same order of magnitude as the cost of a reverse osmosis plant.

8.3.2 Operating costs

Operating costs consist primarily of manpower, chemicals and power requirements. As far as manpower is concerned, and in the absence of data on plant operation over a long period of time, only rough estimates are possible to give (Table 12). The estimated costs for the chemicals used in various types of treatment are shown in Table 13.

Fig. 11.   Capital cost for nitrate removal
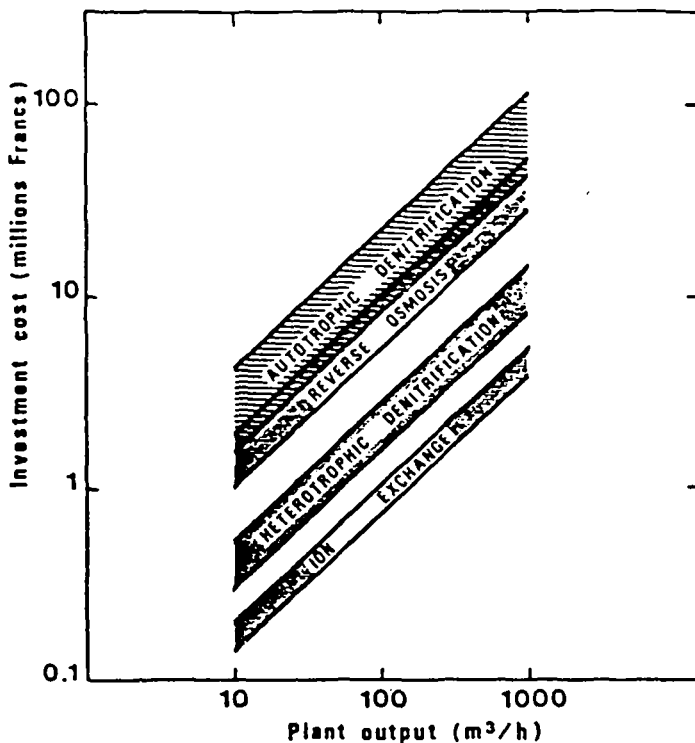
Table 12. Manpower requirements for the main treatment techniques

Treatment	Manpower (months/year)	Remarks
Ion exchange	2.5 - 4	Depends on pretreatment
Denitrification with sulfur	1.5 - 2.5	Comparable to a neutralization treatment
Heterotrophic denitrification	3 - 5	Complete biological treatment
Reverse osmosis	4 - 6	Complex pretreatment

Table 13. Chemical costs for the main treatment techniques

Substrate	Cost of reagents (FF/ton)
Ethanol	6200 - 6500[a]
Acetic acid	3500
Acetone/butanol	4500[b]
Hydrogen	20 000 - 30 000[c]
Sulfur	3000
Brine	250[d]

[a]This cost can drop if ethanol is produced by fermentation.
[b]The use of this substrate, not yet sold commercially, may become of interest in future years.
[c]Cost of hydrogen produced onsite by electrolysis.
[d]Brine containing 300 g NaCl/l.

8.3.3 Effect of nitrate level

Figure 12 shows the costs involved, as a function of the quantity of nitrates removed, for heterotrophic denitrification with ethanol, at a flow rate of 100 m^3/h. The removal of 50 mg/l of nitrates over 10 years doubles the initial investment cost.

Fig. 12. Total cost versus time for a 100 m^3/h plant using ethanol
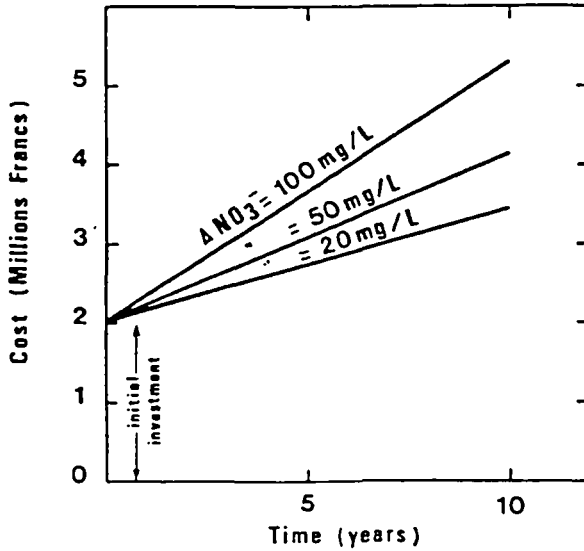
8.3.4 Cost of substrate

This assumes an autotrophic or heterotrophic
denitrification which needs to remove 100 mg/l of nitrates.
Results are presented in Table 14 and indicate that all
chemicals come approximately to the same cost in terms of the
number of electrons given.

Table 14. Cost of chemicals to reduce 100 mg/l of nitrate

Substrate	Cost of reagents alone (FF/m^3 of water produced)
Ethanol	0.29
Acetic acid	0.28
Hydrogen	0.33
Sulfur	0.20

8.3.5 General comparison

The assumption is that the investment is written off linearly over 10 years. The cost of a plant rated at 100 m^3/h, which is required to treat 100 mg/l of nitrates, has been calculated for different processes (Table 15). These figures should be compared with the price of laying pipes, if a better water source were to be used (Table 16).

Table 15. Comparative cost (FF/m^3) of a plant with a capacity of 100 m^3/h for removing 100 mg/l of nitrate

Process	Investment cost	Reagents	Manpower	Total cost
Ion exchange[a]	0.1-0.15	0.50[a]	0.1-0.2	0.7-0.85
Heterotrophic denitrification	0.2-0.35	0.30	0.15-0.25	0.65-0.90
Autotrophic denitrification	1.0-2.80	0.20	0.05-0.10	1.25-3.0
Reverse osmosis	0.7-1.20	-	0.30-0.50	1.0-1.70

[a] Only one half of the resin capacity is assumed to be used for the nitrates, because of the effect of sulfates and carbonates.

Table 16. Comparison of treatment cost versus new adduction[a]

Treatment	Total treatment cost after 10 y (in millions FF)	Equivalent length of pipe (km)	
		Rural area	Urban area
Ion exchange	5.6 - 6.8	20 - 25	6 - 9
Heterotrophic denitrification	5.2 - 7.2	19 - 27	8 - 10
Autotrophic denitrification	10 - 26	37 - 100	13 - 35
Reverse osmosis	8 - 14	30 - 50	11 - 18

[a] The case of a 100 m^3/h treatment unit removing 50 mg/l of nitrate has been used.

Interestingly, ion exchange treatment and heterotrophic denitrification are equivalent to lengths of pipe between 5 and 10 km under roadways and 20 to 30 km in open land. Depending on the problem which arises, these figures will enable decision-makers to draw a circle with this radius, within which better water sources will be possible to find.

8.3.6 Point-of-use treatment and bottled water

The relatively high cost of treating all supplied water raises the question of treating only the volume of water necessary for drinking purposes or, more specifically, for bottle-fed infants.

Small individual reverse osmosis units have been proposed for point-of-use treatment. They are very efficient in removing a wide variety of water constituents. However, they may cause problems of bacterial proliferation and release of organic compounds at trace levels from the membrane itself. Point-of-use treatment could also be performed using ion exchange units, but no experiments have been done so far and the reliability of such a technique is probably no better than that of reverse osmosis. Biological treatments do not appear to be reliable enough on such a small scale. From a general standpoint, all treatments for nitrate removal need improvement as regards reliability and development of methods which control the process automatically. The point-of-use solution, which could also be extended to a wide variety of pollutants, appears economically feasible. However, its maintenance, control and operation may involve sanitary risks which need further investigation.

8.4 Conclusions concerning treatment techniques for nitrate removal

Nitrates can be removed from water by a wide variety of reliable techniques. At present, heterotrophic denitrification seems to be the best treatment. However, the investment costs are so high that it can only be used when relatively large volumes of water need to be treated. For low flows (below 10 m^3/h), denitrification on sulfur seems promising. Point-of-use treatment should also be considered, but its maintenance needs approval by health authorities.

As a general rule, a geological investigation should be carried out prior to any treatment decision. It may shed some light on the origin of the pollutants and lead to simple control measures when the pollution is localized. It will also provide

information on other sources of water and the feasibility of dilution. This remains the simplest treatment of all.

Both physicochemical and biological treatments will not only remove nitrates but will also drastically change the overall composition of the water and may increase treatment costs several-fold. A few plants using either biological denitrification or ion exchange are operating, but data on their cost, maintenance, operational problems and overall efficacy are inadequate.

Groundwater recharge, or riverbank filtration, may in certain areas offer a way to remove nitrates efficiently, but this process also requires much more systematic scientific evaluation.

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9. CONCLUSIONS AND RECOMMENDATIONS

9.1 Conclusions

1.   The use of different units to express nitrate levels in various media, including water, is unsatisfactory and uniformity is needed.

2.   Levels of nitrate in water sources used for drinking-water supply are increasing, especially the levels in groundwater supplies.

3.   Increasing use of artificial fertilizers, disposal of waste (particularly from animal farming) and changes in land use are the main factors responsible for the progressive increase of nitrate levels in water supplies.

4.   For most people in Europe, drinking-water contributes no more than 30% of the total dietary intake of nitrates. The main source of dietary nitrate is vegetables.

5.   Drinking-water with a nitrate concentration at or lower than the present WHO guideline value of 10 mg/1 of NO_3-N is of satisfactory quality for bottle-fed infants.

6.   There is no convincing evidence of a relationship between gastric cancer and consumption of drinking-water containing nitrates at or below the present guideline value; above this level the evidence is inconclusive.

7.   Satisfactory nitrate removal from drinking-water will present serious technical and financial problems to water undertakings.

9.2 Recommendations

1.   The WHO guideline value for nitrate of 10 mg/1 of NO_3-N, based on consideration of the risk of methaemoglobinaemia in bottle-fed infants, is endorsed. In areas where this value is exceeded, provision of low-nitrate drinking-water for infant feeding should be considered.

2. Descriptive epidemiological studies relating the incidence of, or mortality from, gastric cancer in whole populations to various estimates of nitrate exposure are comparatively weak tools for demonstrating cause-and-effect relationships. Efforts

should be made to measure total nitrate intake in individuals in future studies of this type.

3. Assessment of possible hazards in foodstuffs and water requires an improvement of analytical methods to enable study of the relationship in vivo between nitrate intake and nitrosation.

4. Intervention studies should be carried out in groups at high risk for gastric cancer, specifically to assess the effect of nitrosation inhibitors such as vitamin C.

5. Special attention should be paid to water pollution control measures in order to decrease the nitrate load originating from direct discharges and nonpoint sources and also to reduce nitrogen levels in sewage treatment plant effluent.

6. Efforts should be made to minimize the contamination of water supplies by nitrates originating from agricultural practices.

7. Future efforts to develop the technology of nitrate removal from drinking-water should be coordinated, and treatment costs should be taken into consideration. Simple treatment processes, such as biological denitrification on sulfur, which could be applied in small areas or in less-developed countries, should receive special attention.

8. Further studies are required on denitrification processes in rivers, lakes and reservoirs.

9. Hydrogeological investigations should be carried out prior to any decision on treatment requirements. This type of investigation may shed light on the origin of nitrates and lead to simple control measures when the pollution source is localized.

10. Nitrate problems in groundwater in localized aquifers might well be overcome if further investigation were encouraged into the technique of reduction in situ in the ground. This investigation should be pursued.

11. For scattered populations dependent on shallow wells, a low-nitrate bottled water supply may be a feasible alternative to water treatment. The overall costs of such an alternative should be investigated. Such water, provided mainly for infant feeding, should be supplied in small containers to reduce the hygienic risks.

12. Various analytical methods are available for the determination of nitrate in drinking-water. However, it is fundamental that analytical quality assurance, such as interlaboratory calibration exercises, be carried out regularly to ensure the reliability and comparability of data.

13. Nitrate concentrations in rain and snow in the European Region are increasing. The phenomenon should be investigated in relation to nitrate levels in drinking-water sources.

A NOVEL APPROACH TO NITRATE-INDUCED
METHAEMOGLOBINAEMIA RISK ESTIMATION

This alternative approach to nitrate-induced methaemoglobinaemia risk estimation was proposed at the meeting by Professor P. Grau. It is based on haemoglobin (Hb) – methaemoglobin (MetHb) transfer stoichiometry. An assumption is made that all nitrate consumed by the infant would be reduced to nitrite in the gastric tract. Based on reported lethal doses of sodium nitrite for 60-kg adults (1.6-9.5 g), a dose corresponding to 65% of MetHb may be taken to be 2.5 g of $NaNO_2$. Other assumptions made in the calculation are:

- blood volumes - 60-kg adult, 6 l; infant, 0.4 l;
- Hb concentration - adult, 16% of HbA; infant, 12% of HbF;
- lethal proportion of MetHb - adult, 65%; infant, 50%;
- rate of MetHb reduction - adult twice as fast as the infant due to enzyme activity; and
- infant toxic level - 10% of MetHb.

Lethal concentration calculation

$$C_L = 2.5 \times \frac{N}{NaNO_2} \times \frac{IV}{AV} \times \frac{HbI}{HbA} \times \frac{IfD}{AfD} \times 0.5 \times 10^{-3}$$

$$C_L = 2.5 \times \frac{14}{69} \times \frac{0.4}{6} \times \frac{12}{16} \times \frac{50}{65} \times 0.5 \times 10^{-3} = 9.75 \text{ mg of } NO_3\text{-N}$$

Toxic concentration

$C_T = 1.95$ mg NO_3-N

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