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SUMMARY AND EVALUATION

This integrated criteria document "Nitrate" contains data on nitrate for the purpose of an effect-directed environmental policy. Insofar as required, other nitrogen compounds have also been included. Attention will successively be paid to the physicochemical properties and existing standards, sources, emissions, dispersion and conversion, concentrations occurring in the environment, effects on man and the environment, measuring methods and possibilities of reducing the emissions. The financial aspects will also be considered.

Nitrates play an important role in nature in the protein synthesis of higher plants and, more generally, an essential role in the nitrogen cycle in nature. High concentrations do not occur naturally because the nitrate that becomes available from mineralization is directly taken up by plants. However, large emissions occur as a result of human activities, from industry, agriculture and livestock farming, households, traffic and combustion processes. For example, the annual direct emission into surface water, soil and air is 50, 395 and 358 ktonnes of nitrogen, respectively. This does not include the approximately 890 ktonnes of nitrogen present in waste and dredging spoil, but uptake by plants, manuring and grassland losses have been taken into account. Of the volume of nitrogen emitted into the air, 80 ktonnes find their way back to the soil via deposition, as well as 72 ktonnes coming from across the borders. On the other hand, 278 ktonnes of nitrogen emitted into the air in the Netherlands are exported. Taking into account the occurrence of denitrification in the water-unsaturated zone of the soil, it is estimated that about 300 ktonnes of nitrogen per year end up in the groundwater. The amount of nitrogen which via soil and groundwater reaches the surface water and the drinking water supply indirectly is not known, chiefly because insufficient insight exists into the extent of runoff and denitrification in the groundwater. Of the estimated 200 ktonnes of nitrogen which disappear from the soil through denitrification, at least 11 ktonnes are probably released in the form of NO_x compounds; the remainder escapes as N_2 gas.

The establishment of standards for nitrate in the environment, foodstuffs and drinking water is poorly developed and, where available, generally not well founded. Various models exist for describing the transport of nitrate in soil and groundwater. However, these models have generally not been

validated for the field situation. Uncertainty exists about several things, including the denitrification factor to be incorporated. It depends on many factors. A reasonable insight exists into the relationship between groundwater and denitrification. Little is still known about the effects of the soil conditions. The same is true for the occurrence of denitrification in the groundwater itself. Further research into this as well as into the applicability of existing models is necessary.

Models are also available for describing the dispersion of nitrate in surface water and air. Apart from that, there is insufficient insight into the extent of runoff of N compounds from the soil to the surface water, and of the transport of nitrogen via the groundwater to the surface water. Little is known about the occurrence of organic nitrates in the atmosphere. Wet deposition is an effective process of removing nitrate from the atmosphere. The removal is around $6.7 \text{ kg NO}_3^- \cdot \text{N} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$. Although dry depositions have been calculated at about $17 \text{ kg NO}_3^- \cdot \text{N} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$, no measurement data on this are available. In plants, the uptake of nitrogen in the form of nitrate dominates. Although a great deal is known in plant physiology about how the uptake pattern of cations and anions is influenced by the plant, the techniques to predict and, if required, to regulate relevant factors in the field do not exist.

The occurrence of nitrate in groundwater is strongly determined by the soil conditions; nitrate is not found under anaerobic conditions (peat regions). Under aerobic conditions, the natural background values are $0.2\text{-}0.5 \text{ mg NO}_3^- \cdot \text{N} \cdot \text{l}^{-1}$. As a result of agricultural activities, these concentrations can rise to several tens of $\text{mg NO}_3^- \cdot \text{N} \cdot \text{l}^{-1}$. The large rivers contain naturally nitrate concentrations of up to around $1 \text{ mg NO}_3^- \cdot \text{N} \cdot \text{l}^{-1}$. The concentration in the Rhine is currently about $4 \text{ mg NO}_3^- \cdot \text{N} \cdot \text{l}^{-1}$. For the small surface waters these concentrations vary from less than $0.5 \text{ mg NO}_3^- \cdot \text{N} \cdot \text{l}^{-1}$ in summer to $5 \text{ mg NO}_3^- \cdot \text{N} \cdot \text{l}^{-1}$ or more in winter. Little is known about the occurrence of nitrate in indoor air, although a link with gas appliances has been demonstrated. In the outdoor air, the mean nitrate concentration as aerosols is $3.2 \text{ } \mu\text{g NO}_3^- \cdot \text{N} \cdot \text{m}^{-3}$ and as nitric acid $0.9 \text{ } \mu\text{g NO}_3^- \cdot \text{N} \cdot \text{m}^{-3}$. The concentrations in summer are about 1.5 times higher than during the winter months. As regards food, by far the highest concentrations are found in vegetables. Of the 143 mg of nitrate ($32 \text{ mg NO}_3^- \cdot \text{N}$) taken in on average by man per day via food, 120 mg nitrate ($27 \text{ mg NO}_3^- \cdot \text{N}$) is derived from vegetables. For drinking water from 80% of the groundwater pumping

stations, the daily intake by man via drinking water is less than 20 mg nitrate ($4.5 \text{ mg NO}_3^- \text{-N}$). At 5% of the pumping stations this can rise to 100 mg nitrate ($22.6 \text{ mg NO}_3^- \text{-N}$). Intake via inhalation is less than 0.1 mg nitrate ($0.02 \text{ mg NO}_3^- \text{-N}$).

Nitrate in itself is not very toxic. The principal effects arise as a result of the conversion of nitrate to nitrite. Nitrite is formed from nitrate by bacterial conversion in the oral cavity and/or stomach. Nitrite oxidizes haemoglobin to methaemoglobin, which interferes with the transport of oxygen by the blood (methaemoglobinaemia). Suitable data concerning the semichronic or chronic exposure of man to nitrate or nitrite on which a no-toxic-effect level can be founded do not exist. As a result, the no-effect level for man will have to be extrapolated from animal experimental data. Since the conversion of nitrate to nitrite in rats differs from that in man, the rat should be regarded as a less suitable experimental animal species for studying the toxicity of nitrate. The no-effect level of nitrate for adult man is therefore calculated from the no-effect level of nitrite in the rat using the percentage of conversion of nitrate to nitrite found for man.

The no-effect level of nitrite in the rat, deduced from a long-term toxicity study, is $10 \text{ mg NaNO}_2 \cdot \text{kg}^{-1} \text{ body wt}$, or $6.7 \text{ mg NO}_2^- \cdot \text{kg}^{-1} \text{ body wt}$. Assuming that the conversion of nitrate to nitrite is actually 5%, this means for adult man a no-effect level of $100\%/5\% \times 10 \text{ mg} \cdot \text{kg}^{-1} \text{ body wt} = 200 \text{ mg NaNO}_3 \cdot \text{kg}^{-1} \text{ body wt}$, or $146 \text{ mg NO}_3^- \cdot \text{kg}^{-1} \text{ body wt}$. Bottle babies of up to 3 months of age are a potential risk group because of a higher conversion of nitrate into nitrite owing to a higher pH in their stomach than in adults. Bacterial gastrointestinal infections can enhance this conversion of nitrate to nitrite. This age group is moreover more susceptible to nitrite-induced methaemoglobin formation because the foetal haemoglobin present is more readily oxidized to methaemoglobin and because the enzyme NADPH methaemoglobin reductase, which catalyzes the reduction of methaemoglobin to haemoglobin, is absent. Assuming that the conversion of nitrate to nitrite is 80% in infants up to 3 months of age, a no-effect level can be calculated amounting to $100\%/80\% \times 10 \text{ mg} \cdot \text{kg}^{-1} \text{ body wt} = 12.5 \text{ mg NaNO}_3 \cdot \text{kg}^{-1} \text{ body wt}$, or $9.1 \text{ mg NO}_3^- \cdot \text{kg}^{-1} \text{ body wt}$. Bottle babies receiving feed made up with drinking water containing $50 \text{ mg NO}_3^- \cdot \text{l}^{-1}$ ingest $8.3\text{--}8.5 \text{ mg NO}_3^- \cdot \text{kg}^{-1} \text{ body wt}$ per day. This means that there is no safety margin between the daily intake of nitrate via drinking water containing this NO_3^-

concentration by infants up to 3 months old and the calculated no-effect level for intake over long periods of time.

The WHO (1985) recommends that, in regions where the drinking water exceeds the standard of $50 \text{ mg NO}_3^- \cdot \text{l}^{-1}$, water with a low NO_3^- -content be supplied for the formula. In the Netherlands, the standard of $50 \text{ mg NO}_3^- \cdot \text{l}^{-1}$ was, according to measurements in drinking water at 255 pumping stations, exceeded at only one station in 1986. True, the concentrations are rising at 62 pumping stations and it is expected that the limit will have been exceeded at at least 9 pumping stations before the year 2000. This is already the case for individual boreholes on the site of the pumping stations. Furthermore, the limit is exceeded at very many private water supplies.

The toxicity of nitrate is also partly determined by the possible formation of carcinogenic N-nitroso compounds (e.g. nitrosamines) in the body through reaction of the nitrite formed from nitrate with nitrosatable compounds that may be present (= endogenous nitrosation). Traces of nitrosamines (dimethylnitrosamine and diethylnitrosamine) have been found in man after the simultaneous consumption of nitrate-rich vegetables and fish (fish contains amines as nitrosatable compounds).

However, it is difficult, if not impossible, to establish in epidemiological studies a cause-effect relationship between exposure to low concentrations of nitrosamines and the incidence of cancer in the general population. Data from a number of epidemiological studies are merely suggestive.

Epidemiological research in persons with gastric lesions or disorders showed that a correlation between the induction of stomach cancer and the oral intake of relatively large amounts of nitrate cannot be ruled out within this group. In persons without gastric lesions or disorders no relationship could be demonstrated between the estimated nitrate intake and the induction of stomach cancer. Tumour incidences were not increased in persons exposed to nitrate or nitrite in the workplace.

For fresh surface water, based on both the results of single-species toxicity studies and recommended values with relation to the eutrophication issue, concentrations of $0.04 \text{ mg NO}_3^- \cdot \text{l}^{-1}$ and $2 \text{ mg NO}_3^- \cdot \text{l}^{-1}$ are considered to be maximally acceptable for oligotrophic and eutrophic waters respectively. These concentrations correspond to 0.01 and $0.50 \text{ mg NO}_3^- \cdot \text{N} \cdot \text{l}^{-1}$ respectively.

A concentration of $0.4 \text{ mg NO}_3^{-1} \cdot \text{l}^{-1}$ ($0.1 \text{ mg NO}_3^{-1} \cdot \text{N.l}^{-1}$) is considered to be maximally acceptable for sea water.

In terrestrial ecosystems, the effects from eutrophication can partly arise as a result of soil acidification. This is especially true of soils naturally deficient in nutrients. Effects on forest ecosystems occur at total nitrogen loads of $20\text{-}30 \text{ kg N.ha}^{-1} \cdot \text{year}^{-1}$ and more. Grassing-up of heathland occurs at total nitrogen loads of $14\text{-}20 \text{ kg N.ha}^{-1} \cdot \text{year}^{-1}$ and more. To maintain a heathland vegetation, effective nitrogen management is therefore essential. For natural grasslands, effects on soil- and entomofauna occur at exposures to total nitrogen of about $50 \text{ kg N.ha}^{-1} \cdot \text{year}^{-1}$. An acceptable nitrogen load for dune ecosystems has not yet been worked out, owing to insufficient quantitative data. A deposition of $5\text{-}10 \text{ kg N.ha}^{-1} \cdot \text{year}^{-1}$ has been proposed as a "safe" value for forest ecosystems. This value can be somewhat higher for a broad-leaved forest than for a coniferous forest, and also provides a reasonable degree of protection to other terrestrial systems.

Numerous methods for determining nitrate exist for each measurement range and for all environmental compartments. Automated methods are preferable. Research into the best methods of preserving samples as well as the encouragement of ring studies is desirable. The development of a measuring strategy is essential for drawing up measurement programmes.

For measurement programmes aimed at soil and groundwater, knowledge of the geohydrological situation is a prerequisite. The measuring frequency chosen depends partly on this and partly on the method and location of sampling, and will roughly vary between one and 4 times per year. In the case of surface water, it is important to make a determination not only of nitrate but also of ammonium, nitrite and organic nitrogen. Only then is it possible to gain an insight into the amount of nitrogen that is potentially available for nitrate formation. A sample should be taken at least once a month. In view of the even dispersion pattern of nitrate in the atmosphere, about 6 measurement sites and a measuring frequency of one 24-hour determination every eighth day is sufficient for determining the wet depositions. As regards the measuring strategy for drinking water, it is not sufficient to take samples of the drawn-off ground- or surface water but it is also necessary to monitor these bodies of water themselves. Considering the fact that the drinking water standard and the recommended value for water for watering livestock are frequently exceeded at private

water supplies, registration and regular sampling of these supplies should be considered. For foodstuffs, it is recommended to sample regularly not only lettuce, spinach and endive but also beetroots, turnip tops and purslane. Special attention to ready-to-eat baby and infant food also seems advisable.

Cutting the nitrate emissions into the surface water by about 80% is possible through, among other things, the modification of sewage treatment plants (the addition of a denitrification step after nitrification). Its cost may amount to 15 guilders per inhabitant equivalent per year. At present, about 35% of the nitrogen present in sewage is removed. Another possible measure is to increase the number of house connections to the sewage treatment plants. Of the 700,000 households not connected, around 20% can probably still be connected at f 15,000 at most per connection.

Insufficient insight exists into the possibilities of treating industrial discharges. As regards emissions from agricultural activities, the nitrogen emission into soil, groundwater and surface water can be reduced through, among other things, better adjustment of the manure dose to the plant's requirement for nitrogen, replacement of animal manure by chemical fertilizer, transport and processing of animal manure, changing the manuring regimen, planting cover crops after the growing season, use of nitrification inhibitors, changing the composition of the livestock feed and increasing the moisture content in the water-unsaturated part of the soil (enhanced denitrification). Application of nitrification inhibitors and changing the manuring regimen are only suitable after the manure dose has been adjusted to the crop's requirement for nitrogen. Replacement of animal manure by chemical fertilizer is only effective if there is a simultaneous reduction in the amounts of animal manure by limitations on production or by manure processing.

Other possible ways of restricting emissions are reducing the application in agriculture of sludge from sewage treatment plants and of sewage sludge, laying out rubbish tips in accordance with the IBC criteria and cutting the nitrogen emission (NO_x and NH_3) into the atmosphere. Through the application of low- NO_x burners and of after-treatment techniques, the emission of NO_x from stationary sources into the air can be reduced by 60 to 90%. A reduction of 50 to 70% is considered feasible for mobile sources through modification of engines. The NH_3 emission from stables can be cut by up to 95% through treatment of the ventilation air. Manure injection or

ploughing the manure into the soil does have a positive effect on the emission into the air (up to about 90% reduction) but, with constant volumes of manure, a negative effect on soil and groundwater.

For purifying drinking water, use can be made of ion exchange, hyperfiltration and biological denitrification. A combination of ion exchange and biological denitrification appears to hold the best prospects for the long-term future. However, the combined process is not yet ready for application in practice. An alternative approach could be denitrification in situ. Various experiments at home and abroad showed nitrogen removal percentages of 50 to 90. There are a few practical applications on a small scale abroad. In the Netherlands, clogging-up problems arose in field tests. These problems were not encountered in trials elsewhere or only to a limited extent.

As regards the financial consequences for the fertilizer industry when environmental measures are taken, the costs will rise by 1% of the turnover, or 3.5% of the value added. As far as relevant agricultural activities such as cattle, pig and poultry farming are concerned, it should be remarked that the endurance of enterprises in this sector is not very great. In this context, it is important to note that the livestock industry is faced with relatively large investments related to the manure problem, which are difficult to absorb because of financial constraints.

INTRODUCTION

Environmental policy at government level is first of all aimed at attaining and maintaining an air quality that assures the health and wellbeing of man and the preservation of animals, plants, goods and ways in which the substance in question is used (Indicative Multi-year Programme (IMP)-Environmental Management 1986-1990).

However, for lack of knowledge it is impossible for the time being to describe fully the overall environmental quality in view. Therefore, attention is now being concentrated on factors which probably entail considerable risks, including environmentally harmful substances. A selection has or will be made of the many substances of relevance, because of emission or use, and a priority list compiled. In principle, so-called integrated criteria documents are drawn up for most of the priority substances.

Integrated criteria documents contain, per substance or substance group, data on the sources and the dispersion pattern (soil, water, air, biotas), the risks based on a weighing-up of exposure concentrations and routes, on the one hand, and harmful concentrations for man, (parts of) ecosystems and materials, on the other, and the technical and economic possibilities of reducing these risks. This information serves as the scientific basis for formulating the effect-directed environmental policy. This may lead to environmental quality standards and a general task-setting for the emission reduction per source type. As a result, the integrated criteria documents are more comprehensive than the criteria documents previously drawn up, which related only to the outdoor air.

The reason for drawing up an integrated criteria document for nitrate was the large emissions into the environment of nitrate and nitrogen compounds that can be converted into nitrate, as well as the clearly detectable effects of nitrate and its derivatives on man and the environment. Although from this angle the compilation of an integrated criteria document "Nitrogen" has also been considered, yet a document specifically concentrating on nitrate was chosen. The properties of nitrate, however, make it inevitable that this document will also frequently discuss other nitrogen compounds, such as ammonium, ammonia, nitrite, NO_x and nitrosamines. The integrated criteria document "Nitrate" should be seen in connection with a separate integrated criteria document on ammonia still to be drawn up.

The layout chosen for the document is as follows: after a description of the relevant properties of nitrate and existing standards (chapter 1), the sources and emissions of nitrate are considered (chapter 2). Next, the dispersion and conversion of nitrate in the environment are discussed (chapter 3) as well as the concentrations found in the environment (chapter 4). Chapter 5 deals with the effects of nitrate and its derivatives, such as nitrite and nitrosamines, on man and the environment. This chapter is actually a summary of a more detailed effect-description, which has been published by the RIVM separately in English, and of the RIN report "Nitrate immission and Dutch ecosystems: a general risk analysis" (in Dutch). The available measuring techniques are reviewed in chapter 6 and the initial impetus is here also given to the development of a measuring strategy. Finally, the possibilities of reducing the emissions and immissions, and their financial consequences, are discussed in chapters 7 and 8. To supplement the general summary, a paragraph "summary and conclusions" is appended to each chapter. In chapter 5 this summary also comprises a risk assessment relevant to the establishment of standards. The summary of chapter 7 recapitulates again the possibilities available for each environmental compartment for reducing emissions, partly based on the data given in chapter 2. The influence on modern agricultural ecosystems has not been considered in this document. Nor have the effects of changes in the manuring regimen been discussed in a quantitative manner.

The concentrations and quantities given in this document are expressed as far as possible in milligrams of nitrate calculated as nitrogen ($\text{mg NO}_3^- \text{-N}$). In view of standards and regulations in force, however, this was not always possible. Therefore, when reading this report, special attention should be paid to the units employed: 1 $\text{mg NO}_3^- \text{-N}$ is equivalent to 4.4 mg NO_3 calculated as nitrate.

The present document was prepared by the RIVM, DHV Consulting Engineers BV, the Netherlands Organization for Applied Scientific Research (TNO), the Institute for Environmental Issues (IvM), the National Institute for Nature Management (RIN), and the Economic Social Institute of the Free University (ESI), under the direction of the RIVM. For some sections, such as pollution-abatement techniques and their cost, valuable information was obtained from several chemical companies. Their valued cooperation was procured through the Office of Environment and Physical Planning of the

Council of Dutch Employers' Unions, VNO and NCW. The section on effects has been submitted separately to experts from industry for comments. The complete document has been checked as to content by a Reviewing Committee of the RIVM. A Supervisory Group comprising staff from the Ministry of Housing, Physical Planning and the Environment, the Department of Inland Waterways/National Institute for Waste Water Purification (RIZA), and the Ministry of Agriculture and Fisheries supervised the preparation of this document.

1. PROPERTIES AND EXISTING STANDARDS

1.1. PROPERTIES

Since the nitrates comprise a group of substances, the properties of nitrate can only be discussed on the basis of common characteristics.

1.1.1. Forms of occurrence

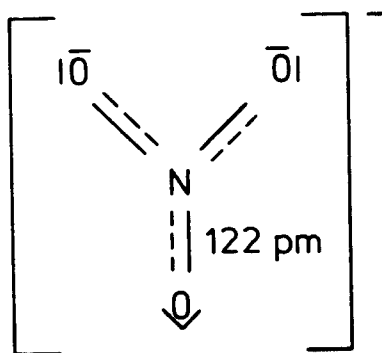
In the biosphere, nitrates form part of a complex system of reactions in which many other nitrogen compounds play a role. The whole system has been summarized under the name of the nitrogen cycle (see chapter 3). Nitrate deposits are relatively rare in the Earth's crust. A major deposit is found in Chile (Chilean nitrate). Artificial fertilizer has been produced from this since the beginning of the 19th century (Bailar et al., 1973). Nitrates for the industrial production of fertilizer and nitric acid are prepared by the binding of atmospheric nitrogen. In the natural nitrogen cycle, ammonium and nitrogen-containing organic compounds are oxidized by bacteria with the formation of nitrates (nitrification).

1.1.2. Physical properties

The common feature shared by all nitrates is the presence of the nitrate ion (NO_3^-). The molecular weight of the nitrate ion is $62.0049 \text{ g.mol}^{-1}$ (Weast and Astle, 1982-'83); 1.00 mg NO_3^- is equivalent to $0.226 \text{ mg NO}_3^- \text{-N}$.

1.1.3. Chemical properties

The nitrate ion has a planar structure and an electron configuration which can be represented as follows (Panel on Nitrates, 1978; Bailar, 1973):



Because of this electron structure, nitrate is a stable anion which does not readily form complexes with metals. Nevertheless, covalent bonds have been demonstrated in some nitrates, e.g. uranyl nitrate, between one of the oxygen atoms of the nitrate group and the metal. The bond with hydrogen and carbon is always covalent (Bailar, 1973). Another consequence of the electron structure of nitrate is that nearly all nitrates are very soluble in water.

1.2. EXISTING STANDARDS AND GUIDELINES

In the Netherlands and abroad, standards and guidelines for nitrate have been established with respect to soil, water, air, food and drinking water.

1.2.1. Soil

For sand and clay-peat, the Environment Programme 1988-1991 (MPV 88, 1987) mentions a target value of 5.6 mg N.l^{-1} for groundwater; the quality of the soil is related to the quality of the groundwater. The limit for groundwater intended for drinking water is $50 \text{ mg NO}_3^{-}.\text{l}^{-1}$ ($11.4 \text{ mg NO}_3^{-}\text{-N.l}^{-1}$). In the IMP-Environmental Management (1986-1990) the target N situation in the soil is for the time being related to the quality of the upper layer of the groundwater. Here, the value of 50 mg of nitrate per litre ($11.4 \text{ mg NO}_3^{-}\text{-N.l}^{-1}$) should be in force and 25 mg per litre ($5.6 \text{ mg NO}_3^{-}\text{-N.l}^{-1}$) in the longer term. The use of animal manures to reduce the phosphate load, and thereby also the nitrate load, is provided for in the Use of Animal Manures Decree (1987).

1.2.2. Surface water

The basic quality for surface water is $44.3 \text{ mg NO}_3^{-}.\text{l}^{-1}$ ($= 10 \text{ mg NO}_3^{-}\text{-N.l}^{-1}$). The Surface Water Contamination Act stipulates a standard of $10 \text{ mg NO}_3^{-}\text{-N.l}^{-1}$ for nitrate. This may only be exceeded where this is the result of natural soil conditions.

In the Order in Council articles 13 and 15 of the Surface Water Contamination Act, a standard of $10 \text{ mg NO}_3^{-}\text{-N.l}^{-1}$ is included for surface water intended for the production of drinking water.

The values given in table 1.1. are included in the Water Board Decree (1984).

No nitrate standards have been set for surface water intended for swimming water, water for salmonidae and cyprinoidea, or shellfish water.

Table 1.1. Standards for the nitrate concentration in surface water intended for the production of drinking water

Quality class Recommended value (A) and limit (B)	I		II		III	
	A	B	A	B	A	B
	-	25	50	50	-	50 mg NO ₃ ⁻ .l ⁻¹
	(	5.6	11.3	11.3		11.3 mg NO ₃ ⁻ .N.l ⁻¹

The quality classes relate to the purification procedure employed in the production of drinking water.

I = simple treatment and disinfection

II = normal physical and chemical treatment and disinfection

III = thorough chemical and physical treatment, refining and disinfection.

1.2.3. Air

Standards for nitrate in air are not known.

Table 1.2. gives standards for a number of countries for NO₂ in the air in view of the formation of NO₃⁻ from NO₂⁻.

Table 1.2. Air standards for NO₂ (µg.m⁻³) in several countries (SCMO-TNO, 1986)

Country	Conc.	Standard	Remarks
The Netherlands	150	limit	24-hour average
	300	limit	1-hour average
	135	limit	98 percentile of 1-hour averages
	175	limit	99.5 percentile of 1-hour averages
FRG	80	limit	annual average
	300	limit	98 percentile of 1/2-hour averages
EEC	50	recommended value	50 percentile of 1-hour averages
	200	limit	95 percentile of 1-hour averages
	135	recommended value	1-hour averages
Switzerland	30	limit	annual average
	80	limit	24-hour average
US	100	limit	annual average
Canada	0-60	target value	annual average
	60-100	recommended value	annual average
	0-200	recommended value	24-hour average
	200-300	limit	24-hour average
	0-400	recommended value	1-hour average
Japan	200-300	limit	1-hour average
	76-115	limit	24-hour average

1.2.4. Food and drinking water

The JECFA set the acceptable daily intake (ADI) for man at 5 mg NaNO_3 and 0.2 mg NaNO_2 per kg body weight (WHO, 1962, 1974).

The following statutory regulations are in force for nitrate in food (Contaminantenboekje, 1987):

Preservatives Decree (Food and Drugs Act): it is not permitted to add nitrate to foodstuffs other than cheese, processed cheese, meat or meat products.

Meat and Meat Products Decree (Food and Drugs Act): no more than 500 mg $\text{KNO}_3 \cdot \text{kg}^{-1}$ and no more than 200 mg $\text{NaNO}_2 \cdot \text{kg}^{-1}$ may be added to meat products.

Infant Food Regulation (Food and Drugs Act): the nitrate content in infant food should not exceed 50 mg $\text{NO}_3^- \cdot \text{kg}^{-1}$ on a dry weight basis.

(Processed) Cheese Decree (Food and Drugs Act): the nitrate content in cheese should not exceed 50 mg $\text{NO}_3^- \cdot \text{kg}^{-1}$, except where otherwise specified in the cheese standards, and that of nitrite should be no more than 200 mg $\text{NO}_2^- \cdot \text{kg}^{-1}$.

In accordance with the Food and Drugs Act (1982), the standards given in table 1.3. have been employed for green vegetables since 1985.

Table 1.3. Standards for nitrate in green vegetables (in mg $\text{NO}_3^- \cdot \text{kg}^{-1}$)

<i>Vegetable</i>	<i>1 April - 1 November</i>	<i>1 November - 1 April</i>
<i>Endive</i>	3500	3500
<i>Lettuce</i>	3500	4500
<i>Spinach</i>	3500	4500

The nitrate standard for drinking water incorporated in the Dutch Water Board Decree (1984) is 11.3 mg $\text{NO}_3^- \cdot \text{N.l}^{-1}$ (target value is 5.6 mg $\text{NO}_3^- \cdot \text{N.l}^{-1}$, in accordance with the EEC Guideline (1980) on the quality of drinking water intended for human consumption). The WHO (1985) recommended value is 10 mg $\text{NO}_3^- \cdot \text{N.l}^{-1}$. These recommended values were established on the basis of health criteria for lifelong consumption. Brief exposure to higher concentrations may be allowed but should be examined case by case, in connection with acute toxicity, among other things.

For water intended for watering cattle, pigs and poultry, the recommended values given are 100, 25 and 25 mg $\text{NO}_3^- \cdot \text{l}^{-1}$ respectively (or 22.6, 5.6 and

5.6 mg $\text{NO}_3^- \cdot \text{N.l}^{-1}$). This is a limit below which the animal runs no risk. The drinking water is unfit for consumption when the nitrate concentration is more than 200, 100 and 50 mg $\text{NO}_3^- \cdot \text{l}^{-1}$ respectively (45.2, 22.6 and 11.3 mg $\text{NO}_3^- \cdot \text{N.l}^{-1}$) (Animal Health Care Foundation). When the concentration in the drinking water lies between these limits, its suitability will depend on concentrations of other parameters (such as NO_2^- and NH_3).

The maximum allowable nitrate intake for the protection of animal health and the maximum allowable nitrate concentrations in coarse fodder are given in the handbook for Cattle Farming (1984). The criterion is that an adult cow may consume per meal (twice a day), without risk, 50 to 60 g of nitrate via soilage and 25 to 30 g of nitrate via hay.

1.3. Summary and conclusions

Nitrate can be a component of several substances and belongs to the much larger group of nitrogen compounds. These compounds can be converted into other nitrogen compounds (nitrogen cycle). Nitrate deposits are rare in the Earth's crust. Nitrates are very soluble in water.

The establishment of standards for nitrate in groundwater is in fact not well-founded and is based on the standards for drinking water. The same is true for surface water. No standards have been drawn up for swimming and fishing waters. Standards for water intended for watering livestock do, however, exist. There are no standards or recommended values for the environmental compartment air. A number of statutory regulations are in force for nitrate in foodstuffs.

It can be concluded that, for food and drinking water, the nitrate standards have been laid down in various statutory regulations. Well-founded standards for the occurrence of nitrate in the various environmental compartments are not available. The values employed are mostly based on drinking water standards.

2. PRODUCTION, APPLICATIONS, SOURCES AND EMISSIONS

In the production, applications, sources and emissions of nitrate, other nitrogen compounds also play a role in principle, but this chapter will discuss only those compounds which make a substantial contribution to the nitrate concentrations in the environment, drinking water and foodstuffs.

2.1. PRODUCTION AND APPLICATIONS

2.1.1. Industry

The production and processing of nitrogen-containing compounds in the industrial sector take place mainly in the chemicals industry and in particular in the fertilizer industry. The fertilizer industry comprises first of all fertilizer plants. However, those factories which produce the basic chemicals intended (almost) exclusively for the manufacture of fertilizers are usually also included. These are the factories for ammonia, nitric acid and phosphoric acid. In general, these factories are also partly interrelated.

The production and sales of fertilizer nitrogen are given in table 2.1. In 1979/1980, the total fertilizer output in the Netherlands was more than 2 million tonnes on a yearly basis (measured in the active constituents N, P_2O_5 and K_2O), 70% of which was exported. Over the past ten years, the production in the Netherlands has followed the trends in world production to an increasing extent, i.e. sharp rises in the production of urea, ammonium nitrate, double superphosphate and mixed fertilizers and a drop in single superphosphate.

However, several new structural developments have become apparent since 1981/1982: the relatively high gas and thus also fertilizer prices and the large increases in capacity in the traditional importing regions. As a result, the total volume of exports has fallen for the first time in 1981/1982, namely, by over 10% (urea, the principal export product, by as much as 35%) in one year. Another, possibly structural, consequence is the increase in imports. For example, the import of calcium ammonium nitrate (the principal fertilizer in the Netherlands) has risen by 60% in one year to a 25% share of the domestic consumption (Handbook on Model Regulations concerning Air Pollution). Since 1979/1980, the consumption of chemical

fertilizers has stabilized both in total volume and the amount applied per unit of surface area, at around 475 ktonnes N.year⁻¹ and about 235 kg N per ha of arable land respectively (Fertilizers no. 2, 1985).

Table 2.1. Production and sales in the Netherlands of fertilizer nitrogen (NMI, 1987) (ktonnes N per year)

	'83/'84	'84/'85	'85/'86
Production	1700	1750	1570
Sales	480	505	500

2.1.2. Agriculture and livestock farming

In agriculture and horticulture, anorganic and organic nitrogen compounds in the form of chiefly chemical fertilizer, animal manure, compost and sewage sludge are added to the soil or, if desired, to nutrient substrate as food for the plant.

The organic nitrogen compounds present in the various fertilizers are converted in the soil by microorganisms first into ammonium compounds (ammonification) and then into nitrate (nitrification; see chapter 3). Ammonium (NH_4^+) and nitrate (NO_3^-) are both taken up by the plants and converted into organic nitrogen compounds (mainly proteins). Part of the nitrogen is taken up by soil organisms and utilized. Part of the nitrate is converted to N_2 by denitrification. This is vaporized into the air (secondary emission). A proportion of the resulting plant products will, insofar that it concerns edible products, be exported from the Netherlands and the remainder will be used for human and animal consumption at home. The greater part of the waste arising from the agricultural and horticultural products will return to the soil either directly or indirectly, i.e. via collection and processing of waste. Livestock feed also contains organic nitrogen compounds. Livestock feed is partly imported and partly produced in the Netherlands. It is converted into meat, other animal products such as milk and eggs, and manure.

The animal products are partly exported and partly intended for domestic human consumption.

The amounts of manure and nitrogen produced are given in table 2.2., divided according to animal species.

Table 2.2. Current production of animal manure and nitrogen(1)
-1
(ktonnes per year ; CBS, 1985)

	Animal manure	Nitrogen(2)	Mineral nitrogen
Cattle (l.m.)(3,4)	78,579	354	177
Feeder calves (l.m.)	1,787	5	4
Pigs (l.m.)	15,926	91	46
Laying hens (l.m.)	1,569		
Laying hens (s.m.)	283		
Broilers (s.m.)	310	31	22
Other poultry (l.m.)	46		
Other poultry (s.m.)	2		
Cats and dogs(5)		12	
Total	98,502	493(6)	249

(1) no precise data are known for sheep, goats, and the like;
the quantities are relatively small

(2) present in the manure after normal storage

(3) one year's production, including feeder steers

(4) l.m. = liquid manure; s.m. = solid manure

(5) VROM/DGMH data

(6) excluding pets, 481 ktonnes.year⁻¹

2.1.3. Households

Nitrogen-containing products are consumed in the home. When preparing food, a proportion ends up with the waste, the remainder is consumed and the greater part again excreted. A small fraction is utilized for the growth of the human body. The excretion is estimated at 3360 grams of N per person per day (STORA, 1985).

2.1.4. Traffic and combustion processes

Combustion processes occur chiefly in:

- power stations, sometimes combined with district heating
- refineries
- chemicals industry
- space heating
- traffic

Nitrogen oxides (NO_x) are produced in the combustion process. One source of NO_x is the combustion of nitrogen-containing components present in fuels,

the so-called fuel NO_x . NO_x can also arise from the nitrogen in the air when the temperature is sufficiently high, the so-called thermal NO_x . The formation of thermal NO_x is mainly determined by the height of the temperature reached in the flame itself and in the total burner zone (in the case of more burners). The reaction rate is low at temperatures below 1400°C .

In particular, the tendency to build increasingly more compact installations with a greater furnace load for higher thermal efficiencies has led to an increase in NO_x emissions.

2.2. SOURCES AND EMISSIONS

The primary emissions of nitrate into water, air and soil are described in this section as well as the primary emissions of other nitrogen compounds insofar as they are relevant to the nitrate concentration in the environment.

2.2.1. Soil

Household and industrial waste water, chemical fertilizer, animal manure in the agricultural sector, and solid waste products are regarded as primary sources of emissions of nitrate and other nitrogen compounds into the soil.

- Household and industrial waste water

The waste water of an estimated population of 300,000 is discharged into the soil via cesspools and septic tanks (DHV, 1987). This corresponds to diffuse discharges totalling 1 ktonne of N per year. The Emission Registration (1974-1984) has observed in industry - the metal industry especially - a limited number of discharges on site. The amount of nitrogen is about 0.002 ktonnes per year. The volume of the discharges ending up in the soil from recreational activities (camping-areas and the like) is not known.

- The use of chemical fertilizer

Chemical fertilizer is put on the soil for the benefit of agriculture and horticulture. This fertilizer contains nutrients for the growth of plants, including nitrogen in various forms. The amount of nitrogen applied to the soil in 1984 is estimated to be 475 ktonnes.

An estimated 75% of this fertilizer nitrogen is utilized in the agricultural production system for plant growth (NMI, 1987). The remainder, 119 ktonnes, is regarded as emission into the soil; of this, around 8 tonnes is re-emitted as ammonia into the atmosphere.

- The use of animal manure

All the animal manure produced ends up on the soil, either directly (during the grazing period) or indirectly, when it is spread on the land.

The amount of nitrogen supplied is unevenly distributed over the Dutch soil. The nitrogen dose via animal manure can vary from 0 to more than 1000 kg N.ha⁻¹.year⁻¹ (KIWA, 1984). It is especially in those regions where intensive livestock farming has developed strongly that the supply of manure is very large. These regions are chiefly the sandy soils of the Gelderse valley, eastern Gelderland, North Brabant (centre and east), northwest Limburg and the south of Overijssel.

A surplus of manure has arisen as a result of the intensification of livestock farming, amounting to 20 to 30 million tonnes per year (IMP-Environmental Management 1986-1990). This surplus is disposed of by manuring more than is necessary for optimal growth of agricultural crops. The manure is spread on the land even outside the growing season, i.e. in the autumn and winter. A large proportion of the nitrogen supplied and also the surplus of the nitrogen not removed by the crop during the growing season will then leach into groundwater (KIWA, 1984).

In 1984, 481 ktonnes of nitrogen in the form of animal manure was introduced into the agricultural system, in this case put onto the land as fertilizer. Of these 481 ktonnes, a proportion escaped during and after manure application as NH₃ into the air, namely, 39 ktonnes of N (see table 2.13.). Of the manure produced in the field, some also escaped as NH₃ into the air, namely, 27 ktonnes (see 2.2.4.), so the actual amount remaining was 415 ktonnes. Part of this would be utilized for plant growth. This is on average 40% under normal conditions (NMI, 1987). This percentage is, of course, lower where there is overuse of manure, but by how much is not known. A utilization of 35% is assumed for the average situation, including overmanuring. The remaining 65%, 270 ktonnes of N, is regarded as emission into the soil.

In addition, when the manure is applied to the soil during periods of frost, an (as yet) unquantifiable percentage of the manure can run off into surface water.

Solid waste

All nitrogen-containing waste matter which (in part) ends up on the ground can in principle be a source of nitrate emission. Table 2.3. lists all the waste products, their nitrogen content and the way they are processed. The quantities are derived from the IMP-Waste Materials 1985-1989, with the exception of those for sewage sludge. The extent of emission from the waste products into the surrounding environment is, apart from the nature of the waste, also heavily dependent on the method of processing and storage and can therefore not be given without further details.

Table 2.3. Nitrogen-containing waste products in the Netherlands

	Wet product (ktonnes per year)	N content (%) (kt)	Diffuse soil (%)	Diffuse water (%)	Diffuse air (%)	Dumping (%)	Inciner- ation (%)
Domestic waste products	4,200(2)	0.6 29	-	-	-	63.5	36.5
Litter from streets, markets and parks, flotsam from canals and harbours	1,000(3)	2(1) 20	x	-	-	x	-
Dredgings, drainage sludge (derived from ditches, ponds, sewers and so on)	400(3) (5% d.m.)	6(1) 1.2	-	-	-	100	-
Production and processing waste (excl. sµg arbeet tare)	2,000(2)	0.3(1) 6	-	-	-	63.5	36.5
Office, shop, services waste	1,500(3)	0.3(1) 4.5	-	-	-	63.5	36.5
Sewage sludge	6,350(2)	- 13	59.8	8.7	-	30.4	1.1
Agricultural waste(4)	500(1) (40% d.m.)	2.0 4	100	-	-	-	-
Dredging spoil	50,000 to 60,000(5) (30% d.m.)	5 825	-	-	-	-	-

(1) estimated

(2) 1984

(3) 1983 (these quantities will have been roughly the same in 1984)

(4) agricultural waste not used as fodder or on the land

(5) of this quantity, an estimated 1/3 is dumped into the sea and 1/3 is stored in repositories (e.g. lagoon); of the remaining 1/3, 20% is dumped on land and 80% into freshwater. In the case of dredging spoil, it is thus mainly a question of dumping it back into surface water.

2.2.2. Surface water

Direct sources of emission of nitrate and other nitrogen compounds into surface water are industrial activities, households, sewage treatment plants and agriculture.

- Industrial activities

The amount of nitrogen in industrial waste water depends on the type of industry. Important industries in this connection are:

- fertilizer industry
- chemicals industry

and to a lesser extent:

- metal industry
- food industry
- fibre and rubber industries

Nitrogen is present in the waste water in the form of both ammonia and nitrate. The amount which is directly discharged into surface water is around 6350 tonnes N.year⁻¹, according to the Emission Registration. The discharges are given in table 2.4. per industry and per type of recipient surface water. Table 2.5. gives the division between nitrate nitrogen and ammonia nitrogen per type of recipient surface water.

Table 2.4. Direct emission of nitrogen compounds from industrial discharges into surface water per industry (ktonnes N per year) (Emission Registration, 1974-1984)

Industry	State water bodies	Non-state water bodies	Total
Fertilizer	2.65	1.80	4.45
Chemicals	1.37	0.15	1.52
Metal	0.26	0.01	0.27
Food	0.01	0.08	0.09
Fibre and rubber	0.01	0.01	0.02
Total	4.30	2.05	6.35

Table 2.5. Annual emission of nitrogen compounds from industrial discharges into surface water, divided into ammonia nitrogen and nitrate nitrogen (ktonnes per year) (Emission Registration, 1974-1984)

	State water bodies	Non-state water bodies
Ammonia nitrogen	2.50	0.15
Nitrate nitrogen	1.80	1.90
Total	4.30	2.05

-Households

In the Netherlands, the content of nitrogen compounds in domestic waste water is 3360 grams of N per capita (STORA, 1985), of which 40% is present in the form of organic nitrogen compounds and 60% as ammonia nitrogen.

In 1983, the waste water of 2,377,000 inhabitants was discharged directly into surface water without treatment, of which 961,000 into state-owned water bodies and 1,416,000 into non-state waters (table 2.6.).

Table 2.6. Direct emissions of nitrogen compounds into surface water by households (ktonnes per year; 1983)(CBS, 1986)

	NH_4^+-N	Org. N	Total N
State water bodies	1.9	1.3	3.3
Non-state water bodies	2.9	1.9	4.8
Total	4.8	3.2	8.1

Part of the pollutants in the domestic waste water ends up in surface water via rainwater overflows from mixed sewer systems. A rough estimate puts this emission at 0.62 ktonnes of N per year (Feenstra and Van der Most, 1985).

- Sewage treatment plants

The waste water of 16,014,000 inhabitant equivalents (i.e.) was treated in sewage treatment plants (STPs) in 1983, namely, 11,320,000 i.e. coming from households and 4,694,000 i.e. from industries (CBS, 1986).

A total of 64.5 ktonnes of nitrogen was supplied to the STPs in 1983, consisting of around 60% ammonia nitrogen and 40% organic N compounds (CBS, 1986). Nitrogen compounds can undergo a number of conversions in sewage treatment plants, which affect both the total amount and the type of nitrogen compounds discharged into surface water:

- removal of organic N compounds with primary sludge in (pre-)settling tanks
- incorporation of organic N compounds into ammonia nitrogen (ammonification)
- conversion of ammonia nitrogen to nitrate nitrogen (nitrification)
- conversion of nitrate nitrogen to elementary nitrogen (denitrification)

Nitrification and denitrification occur only in certain types of treatment plants (see section 7.2.).

The nitrogen carried off with the effluent of the STPs is chiefly in the form of ammonia- and nitrate nitrogen.

A breakdown of the discharges per type of recipient surface water is given in table 2.7.

Table 2.7. Discharge of nitrogen compounds by STPs in 1983 according to type of recipient surface water (ktonnes N per year)

Manager	Type of recipient surface water	Total N
Dept. of Public Works	salt state-owned water	4.1
	large rivers	6.1
	canals	2.4
	lakes	0.3
Non-state	polder water	5.1
	running water	10.5
	canals	5.2
	lakes	0.1
Total		33.8

In 1983, the STPs under local government control carried off 4,381,000 m³ of sewage sludge, equivalent to 202 ktonnes of dry matter containing 9.7 ktonnes of N (CBS, 1986).

Private companies operated 258 treatment plants in 1983 with a total capacity of 6,800,000 i.e. (Department of Public Works, 1984).

In 1983, 59 private sewage plants were surveyed, with a capacity of 3,900,000 i.e. and a load of 3,000,000 i.e. (77%). These plants produced 541,000 m³ of sewage sludge in 1983, equivalent to 94 ktonnes of dry matter (17.4%). The high content of dry matter is probably explained by the employment of sludge-dewatering systems in these treatment plants. The dry matter in this sludge contained on average 1.7% N, equivalent to a total of 1.6 ktonnes of N, of which 4 ktonnes were sold to the agricultural sector (Martens, 1986). Assuming a similar load for the 199 treatment plants not surveyed with a proportionately similar production of dry matter, then 70 ktonnes of dry matter containing 1.2 ktonnes of N were derived from these treatment plants.

Assuming a dry matter content of 6%, these treatment plants produced 1,400,000 m³ of sludge.

A total of 6,100,000 m³ of sewage sludge was thus produced in 1983, equivalent to 366 ktonnes of dry matter and containing 12.5 ktonnes of N. In view of the increase in previous years it is expected that 6,350,000 m³ of sewage sludge will have been produced in 1984, containing around 13 ktonnes of N.

- Agriculture

The direct discharge of manures into surface water is prohibited. An as yet unquantifiable amount can end up indirectly in surface water via runoff.

2.2.3. Air

Sources of emission of nitrate and other nitrogen compounds into the air are industrial activities, combustion processes and agricultural activities.

- Industrial activities

Apart from combustion processes, the manufacture, storage and transfer of fertilizers are the only major activities whereby nitrogen compounds are being released. The emissions are derived from 7 fertilizer-producing companies and 12 storage and transshipment companies. Table 2.8. gives the amount emitted per substance.

Table 2.8. Emissions of nitrogen-containing compounds into the air resulting from the production, storage and transfer of fertilizers (tonnes per year) (Emission Registration 1974-1984; RIVM, 1985)

	Emission course dust	Emission fine dust	Total	Total N
Mixed fertilizers	130	470	600	ca 62
Nitrogen-containing fertilizers	145	3065	3210	ca 636
Total	275	3535	3810	ca 698

- Combustion processes

The Dutch emissions of NO_x are given per source in table 2.9. (Statistical Pocket Book, 1986)

Table 2.9. Emissions of NO_x into the air in 1984 and 1985 (ktonnes N)

	1984		1985	
	kt NO _x	kt N	kt NO _x	kt N
<u>Stationary sources</u>				
1. Power stations and district heating	82	24.6	83	24.9
2. Refineries	21	6.3	19	5.7
3. Industry	42	12.6	45	13.5
4. Space heating	31	9.3	33	9.9
5. Others	11	3.3	12	3.6
Subtotal	187	56.1	191	57.6
<u>Mobile sources</u>				
6. Road traffic	270	81	-	-
7. Inland shipping	22	6.6	-	-
8. Agricultural machines	23	6.9	-	-
9. Others	12	3.6	-	-
Subtotal	327	98.1		
Total	514	154.2		

- Agricultural activities

The main cause of emissions of nitrogen compounds, in this case ammonia, into the air is the decomposition of animal manure and chemical fertilizer. The emissions of NO_x from combustion processes in the agricultural sector, namely, when agricultural machines are heated and used, have been included under "combustion processes".

In 1982, the decomposition of chemical fertilizers yielded an ammonia emission of 7.5 ktonnes of N from the consumption of around 450 ktonnes of N present in the fertilizer (Buysman et al., 1986). On the basis of this emission factor and the consumption figures, emission in 1984 is estimated to be 8 ktonnes of N as ammonia.

Emission of ammonia from animal manure occurs:

- during the (temporary) storage of manure and ventilation of the barn;
- during and some time after application of the manure to farm- and grasslands;
- immediately after manure production in the field (during the grazing period).

Variables connected with ammonia emission

The extent to which animal manure releases ammonia depends on a number of physical and chemical parameters, the principal ones being:

- total nitrogen content and ammonia fraction in the manure (N_t);
- acidity (pH) of the manure;
- temperature of the manure;
- atmospheric conditions.

In addition to the above-mentioned parameters, a number of factors which can be influenced directly are also relevant:

- conditions in the barn (manure surface area; ventilation system; manner of manure storage and manure treatment; the degree of filthiness of the manure storage rooms and animal housing);
- whether or not the manure is injected or ploughed into the soil during and after its application.

Total ammonia emission from animal manure in the Netherlands

At present, research is under way into all aspects of the ammonia emission, the results of which are expected to become available at the end of 1988.

Only for the ammonia emission from barns have very recent factors been stated in the Ecology Guideline of the Nuisance Act (1986).

The discussion has been confined to the data available in the literature, especially since an integrated criteria document on ammonia is still to be drawn up.

- Ventilation of barn air

The ammonia emission from stables (see table 2.10.) has been calculated using emission factors for the barn emission for each kind of animal (DGMH, 1987) and counts for the month of May 1984 (agricultural statistics).

Table 2.10. Emissions of ammonia from the stable, including manure storage, in 1984

Kind of animal	NH ₃ (ktonnes per year)
Dairy cows and brood cows	24.5
Heifers <2 years	6.6
Veal calves	1.0
Beef heifers and steers	0.5
Brood sows (incl. piglets)	8.8
Breeding sows (<7 months)	0.2
Breeding sows (>7 months up to the first service)	0.8
Stud boars	0.2
Porkers/breeding boars	11.6
Broiler parent animals	1.1
Broilers	5.2
Breeding chickens (broiler parent animals)	0.4
Breeding chickens (laying hens) liquid manure	0.5
Breeding chickens (laying hens) dry manure	1.1
Laying hens liquid manure	2.1
Laying hens dry manure	4.9
Total	69.5 ktonnes

The total nitrogen emission from the barns is thus 57 ktonnes of NH₃-N per year.

- Manure storage

At present, emission factors for manure storage facilities are not available.

- Application of manure

The total ammonia emission can be estimated from emission factors during manure application. Results of emission measurements are not yet known (see table 2.11.) (Buysman et al., 1985).

Table 2.11. Ammonia emissions during and after application of manure (ktonnes N per year)

Kind of animal	NH ₃
Cattle	23.9
Feeder calves	1.2
Pigs	8.0
- porkers	
- others	
Poultry	5.7
Total	38.8

- Grazing period

Grassland is manured directly during the grazing period of cattle (about 180 days a year). The emission factors for urine and faeces are assumed to be 40% and 5%, respectively, of the N_t content (Buysman et al., 1985). As regards the Dutch situation, a few preliminary results are available (Klarenbeek and Don, 1985). These measurements showed that the emission during the grazing period is about 21.1 kg of NH₃ per unit of cattle. Assuming a nitrogen production of 15.1 kg $N_t \cdot \text{year}^{-1}$ from faeces and 18.1 kg $N_t \cdot \text{year}^{-1}$ from urine, this means that about 6.4% of the N_t content of the total amount of manure produced in the field is emitted as ammonia. The total ammonia emission during the grazing period is 72 ktonnes NH₃·year⁻¹ or 59 ktonnes N·year⁻¹ according to the measurement results, and 33.1 ktonnes NH₃·year⁻¹ or 27.3 ktonnes N·year⁻¹ according to estimates using emission factors.

To be on the safe side, an emission factor of 27 ktonnes of N per year is used for the calculations.

2.2.4. Depositions and cross-border emissions

A proportion of the nitrogen compounds emitted in the Netherlands will be transmitted abroad via air and water, just as a proportion of the nitrogen compounds released abroad will enter the Netherlands.

Import and export via soil and groundwater are here not taken into account in view of the low transport rate and the local impact.

- Air

Full data on the import and export of nitrogen compounds via the air are not available.

The following can be deduced from the deposition balance sheet of NO_y and NH_x (Erisman et al., 1987) (situation 1985):

NO_y : emission in the Netherlands	160 ktonnes N
total deposition in the Netherlands	<u>83</u>
net export	77
emission in the Netherlands	160 ktonnes N
deposition of Dutch emissions	<u>29</u>
export of Dutch emissions	131
deposition on the North Sea	18 ktonnes N
NH_x : emission in the Netherlands	198 ktonnes N
total deposition in the Netherlands	<u>69</u>
net export	129
Emission in the Netherlands	198 ktonnes N
deposition of Dutch emissions	<u>51</u>
export of Dutch emissions	147

No allowance was made for an additional emission into the air resulting from a basal denitrification of about 20% of the nitrate present in the water-unsaturated zone. This basal emission is about $189 \text{ ktonnes N} \cdot \text{year}^{-1}$ in the form of N_2 and, in part, also nitrogen oxides. According to Dowdell (1982) at least 6% of this nitrogen (11 ktonnes) will be emitted in the form of NO_x , while the remainder will escape as N_2 .

- Surface water

The large rivers account for the greater part of the import and export of nitrogen compounds via the surface water. The import via the large rivers is given in table 2.12. Since the effect of the Westerscheldt on the surface water of the Netherlands is believed to be very small, the data on this have not been included in the table.

Table 2.12. Importation of nitrogen compounds via the Rhine and the Meuse (ktonnes N per year) (Dept. of Public Works/RIVM, 1980-1985)

	<u>Rhine</u>		<u>Meuse</u>	
	nitrate	tot. nitrogen	nitrate	tot. nitrogen
1980	313	466	25	36
1981	371	537	31	46
1982	408	549	23	34
1983	320	451	26	38
1984	339	501	32	48

The export of nitrogen compounds via surface water is given in table 2.13.

Table 2.13. Exportation of nitrogen compounds via surface water per year, for the years 1980 and 1981 (ktonnes N per year) (RIZA/RWS, 1983)

	<u>Wester- scheldt</u>	<u>Nieuwe Waterweg</u>	<u>Haring- vliet</u>	<u>Noordzee kanaal</u>	<u>IJssel- meer</u>	<u>Eems- Dollard</u>	<u>Oude Rijn</u>
NO_3^- -N	24	177	135	8	37	9	1
Kjeldahl-N	25	73	44	12	29	21	1
Total	49	250	179	20	66	30	2

2.3. Summary and conclusions

In view of the interactions between nitrogen compounds, it is not advisable to limit this chapter to nitrates. Other nitrogen compounds which can contribute to the nitrate burden should also be included in the consideration.

A summary of all relevant emissions is given in table 2.14. It shows clearly that the emissions into the soil predominate, particularly from fertilizers (chemical fertilizer and animal manure) and waste materials. Regarding the emissions into the air, traffic and industrial combustion

processes (NO_x) are also of particular significance as well as livestock farming. One striking feature is that for the environmental compartment air the export of N compounds far exceeds their import. Indirectly, the Netherlands has a large import surplus of N compounds as a result of the importation of raw materials for chemical fertilizers, fuels and livestock feeds.

An attempt has been made in figures 2.1. to 2.5. inclusive, for the major nitrogen sources, to provide some insight into the nitrogen flows. It is clear that with respect to ground, surface and drinking water, in particular, an adequate numerical basis is lacking.

The possibilities of reducing the emissions will be discussed in more detail in chapter 7.

Table 2.14. Direct emission in 1984 (ktonnes N per year)(1)

Source	Soil	Water	Air	Total
Chemical fertilizer	111(4)	5	8.7(8)	125
Other industries	-	2	-	2
NO_x	-	-	154	154
Livestock farming	270(5)	-	123(8)	393
Households	1	9(3)	-	10
STPs	13(2)	34(3)	-	47
Waste materials(10)	65	-	-	65
Dredging spoil	60(6)	840(9)	-	900
Import(7)	n.a.	549	72	
Export	n.a.	596	278(8)	

(1) in addition to the direct emissions, there is also deposition from the air. This is on average 42 kg N per ha per year (27.6 kg of dry deposition and 14.4 kg of wet deposition). Total deposition in the Netherlands amounts to 152 ktonnes N per year

(2) sewage sludge

(3) 1983

(4) taking into account 75% plant uptake and 8 ktonnes of air emission

(5) taking into account 35% plant uptake

(6) including 60 from dredging spoil; the percentage that actually leaches from the soil is not known

(7) Rhine and Meuse only

(8) not taking into account the effects of denitrification in the water-unsaturated zone

(9) of this, 300 ktonnes is dumped into the sea, 240 ktonnes into surface water and 300 ktonnes is stored in repositories (e.g. lagoon)

(10) excluding sewage sludge (see STPs)

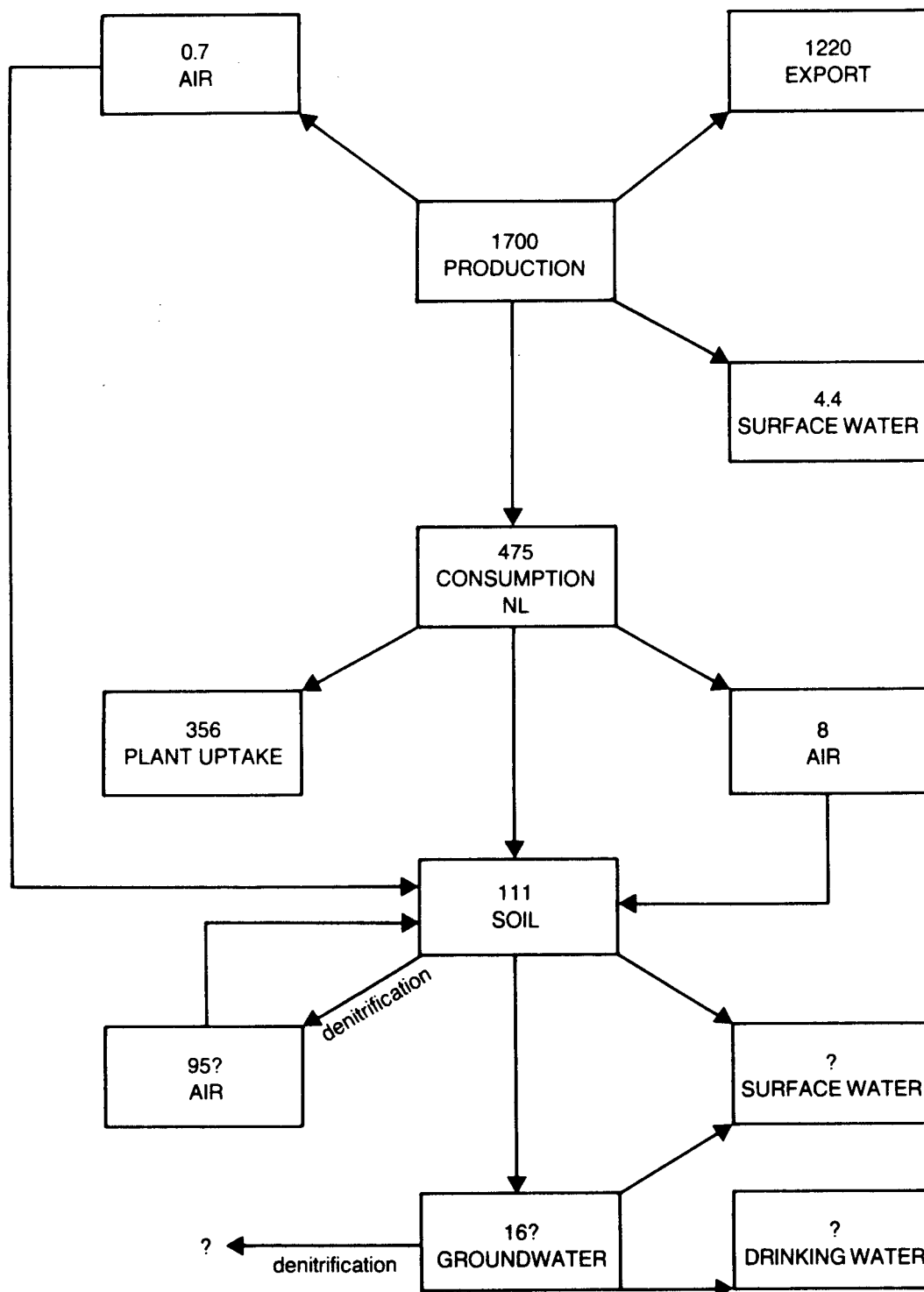


Figure 2.1. N balance sheet of chemical fertilizer, ktonnes per year

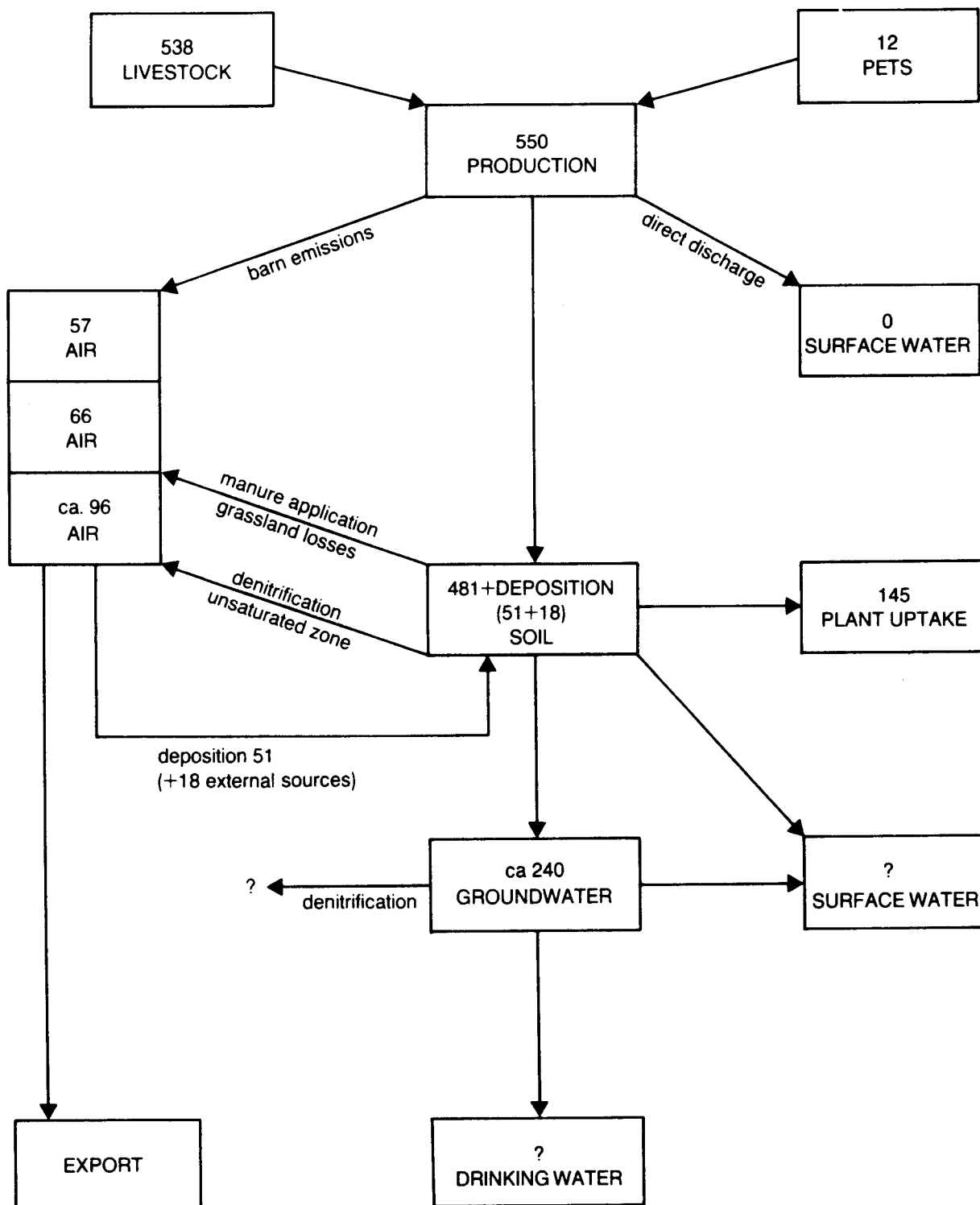


Figure 2.2. N balance sheet of animal manure, ktonnes per year

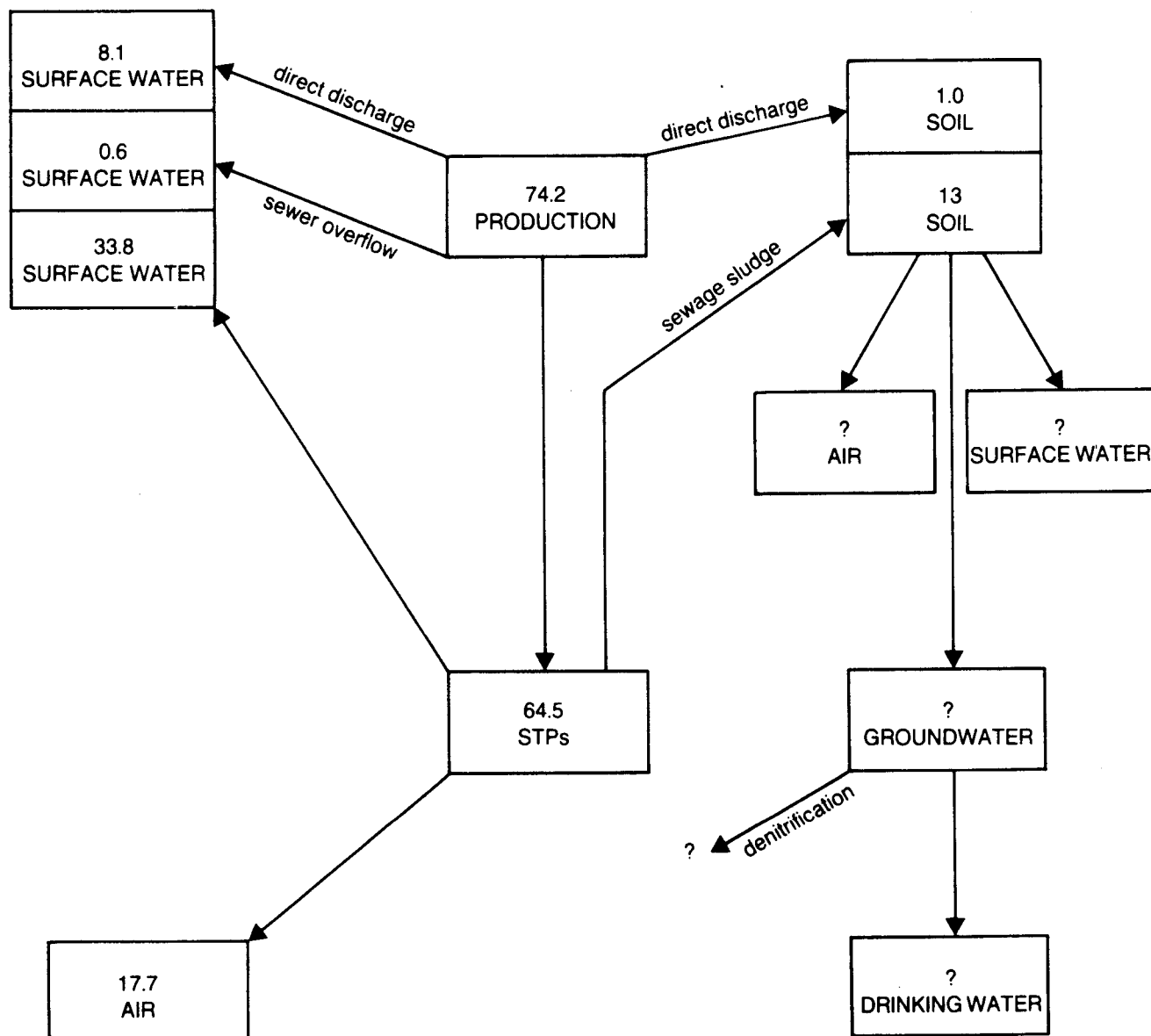


Figure 2.3. N balance sheet of household and industrial waste water, ktonnes per year

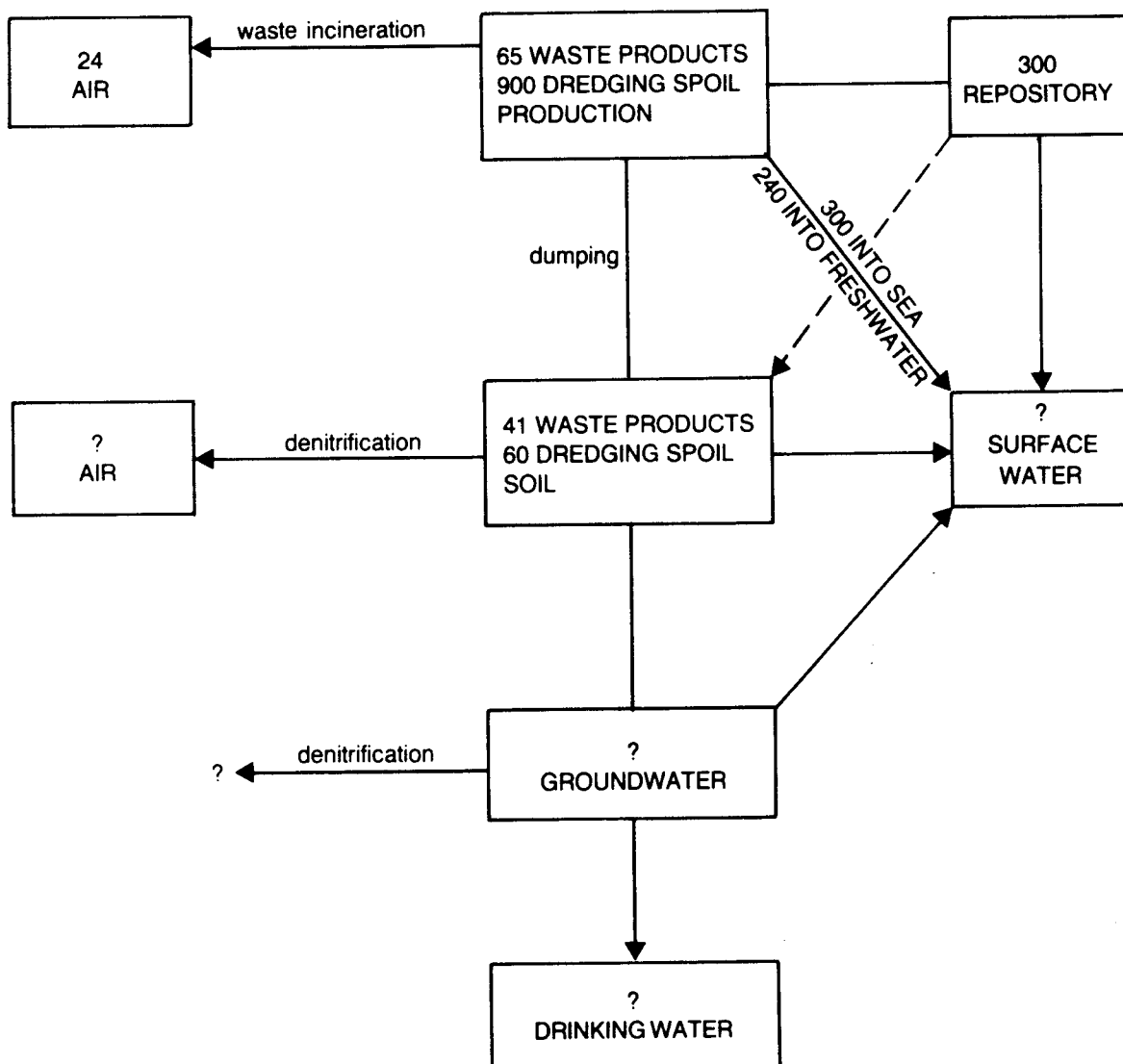


Figure 2.4. N balance sheet of waste materials, ktonnes per year (excl. sewage sludge)

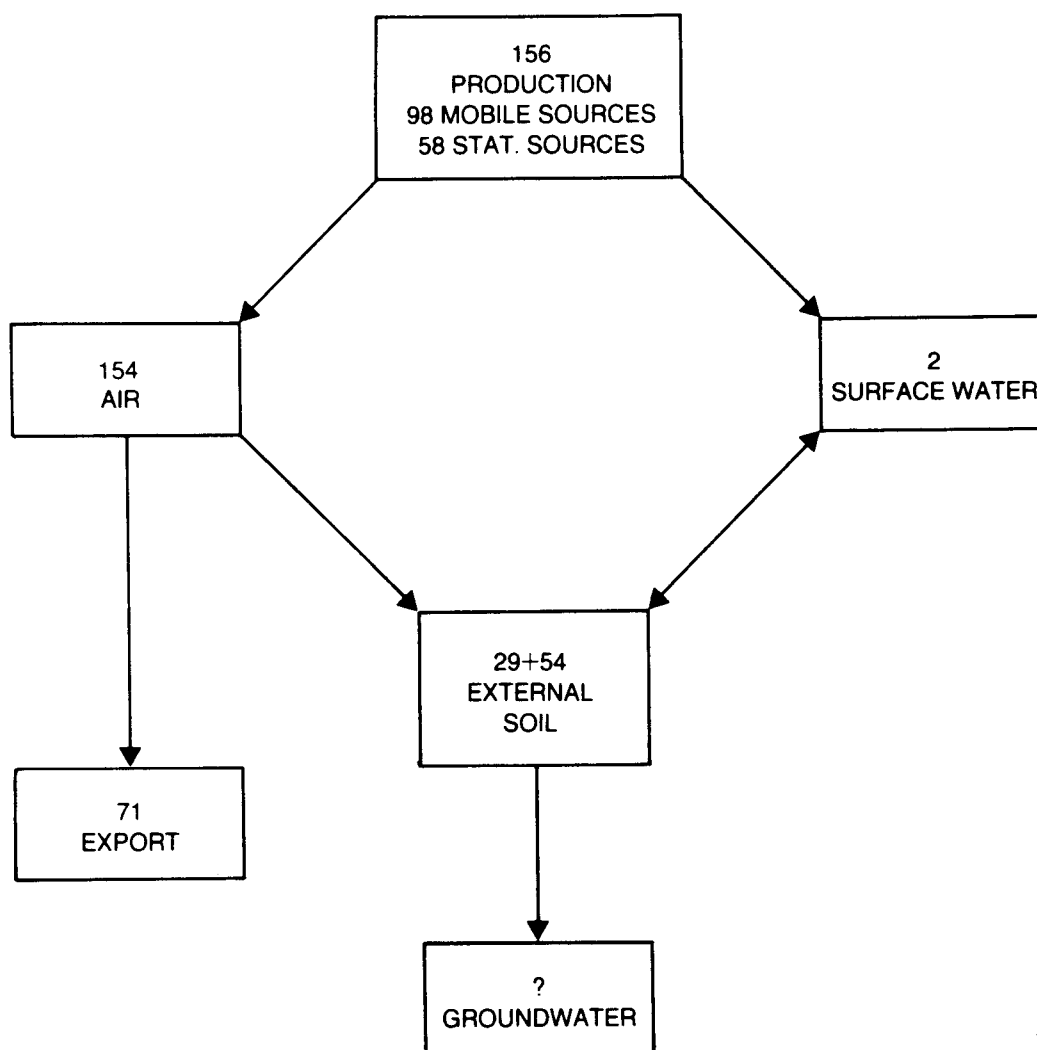


Figure 2.5. N balance sheet of industrial emissions, ktonnes per year
(excl. chemical fertilizer manufacture)

3. DISTRIBUTION AND TRANSFORMATION

3.1. BEHAVIOUR IN SOIL

3.1.1. Distribution

Nitrate does not bind to the soil particles and can therefore leach into groundwater or enter surface water via runoff. The extent to which this occurs depends on the amount of nitrogen applied, plant uptake, the soil conditions and the processes of ammonification, nitrification and denitrification occurring in the soil (see 3.1.2.). Once nitrogen has penetrated into the groundwater it is carried off in it and can only be removed from it by denitrification under the right circumstances.

In general, the losses due to leaching from farmland are greater than from grassland. This is because of the continuous mineralization of the humus after harvesting, the mineralization of the root residues left in the soil and the absence of a crop that can absorb the mineral nitrogen formed. In view of the hydrological and soil conditions (e.g. the extent to which aerobic processes can occur), leaching from sandy soils will be greater than from clay and peat soils. Tables 3.1. and 3.2. give a picture of the nitrate leaching related to the manure dose, land use and soil type, taken from the report of the Wiggers Commission (1986). The water table is also important for the extent of leaching. The least leaching occurs with high water tables because of enhanced denitrification and more possibilities for uptake by plants. Table 3.3. gives an impression of this (Steenvoorden, 1983).

Table 3.1. Calculated nitrate leaching at a depth of 1 metre below ground level (g.l.) resulting from the application of chemical fertilizer, for grassland and farmland on sandy, clay and peat soils with a water table of about 1 m -g.l. The results show the additional leaching as compared to no fertilization(1)

<u>In kg N per ha per year compared to no fertilization</u>					
GRASSLAND (not grazed)					
N fertilization	<u>100</u>	<u>200</u>	<u>300</u>	<u>400</u>	<u>500</u>
Leaching from sandy soil	0	4	25	56	120
Leaching from clay soil	0	2	7	15	25
Leaching from peat soil	0	2	4.5		
FARMLAND					
N fertilization	<u>100</u>	<u>200</u>	<u>300</u>		
Leaching from sandy soil	25	50	75		
Leaching from clay soil	7	18	29		
<u>In % of the chemical fertilizer N applied</u>					
GRASSLAND (not grazed)					
N fertilization	<u>100</u>	<u>200</u>	<u>300</u>	<u>400</u>	<u>500</u>
Leaching from sandy soil	0	2	8	14	24
Leaching from clay soil	0	1	2	5	5
Leaching from peat soil	0	1	1.5		
FARMLAND					
N fertilization	<u>100</u>	<u>200</u>	<u>300</u>		
Leaching from sandy soil	25	25	25		
Leaching from clay soil	7	9	10		

(1) The annual leaching with no fertilization (base load) is about 5 kg N per ha for grassland on sandy and clay soils, 0 kg N per ha for peat soil, and for grassland it is about 45 kg N per ha on sandy soil and 25 kg N per ha on clay soil (Kolenbrander, 1981).

Table 3.2. Nitrate content in the groundwater at a depth of 1 metre below ground level with no fertilization, and the increase in content as a result of fertilization by chemical fertilizer N. The groundwater receives 300 mm of drainage water per year. The data are adapted from table 3.1.

<u>Increase in relation to no fertilization</u>						
GRASSLAND						
N fertilization (kg N per ha per yr)	<u>0</u>	<u>100</u>	<u>200</u>	<u>300</u>	<u>400</u>	<u>500</u>
Contents below sand (g N per m ³)	2	0	1	8	19	40
Contents below clay (g N per m ³)	2	0	0.5	2	5	8
Contents below peat (g N per m ³)	0	0	0.5	1.5		
FARMLAND						
N fertilization (kg N per ha per yr)	<u>0</u>	<u>100</u>	<u>200</u>	<u>300</u>		
Contents below sand (g N per m ³)	15	8	17	25		
Contents below clay (g N per m ³)	8	2	6	10		

Table 3.3. Influence of the springtime water table (m -g.l.) on nitrate leaching from farmland on sandy soil, fertilized during the autumn with 1,900 kg N per ha per year in the form of semiliquid pig manure

Springtime water table	Annual leaching of nitrate (kg N per ha)	Leaching in relation to dose (%)
1.5	740	39
1.0	510	27
0.5	95	5

The time of fertilizer application is also important for the extent of leaching, which is related to plant uptake. Nitrogen added to the soil shortly after the growing season will often be situated below the root zone at the onset of the next growing season and can thus no longer be taken up by the plants (see table 3.4; Lammers, 1983, 1984).

Table 3.4. Nitrate leaching from farmland and grassland calculated for the situation in which denitrification does not occur (A) or does occur (B) below the root zone. The figures apply to semiliquid cattle and pig manure (percentages of total N)

Time of fertilizer application	Farmland				Grassland	
	sand		clay		sand	
	A	B	A	B	A	B
October	40	20	27	14	17	9
November	37	18	25	13	20	10
December	32	16	17	9	10	5
January	22	11	12	6	3	2
February	14	7	8	4	0	0
March	6	3	4	2	-	-

An illustration of the relationship between nitrogen dose and leaching from farmland and grassland is given in figure 3.1., taken from Kolenbrander (1981).

In addition, several empirical and process-describing models have been developed in recent years, describing the leaching of nitrate into groundwater and the transport of nitrate in the groundwater. However, these models have generally not (yet) been validated for the field situation. For example, recent research at the RIVM and the State University of Utrecht (De Wit and Bleuten, 1987) shows that the leaching of nitrate from grassland with lower water tables (groundwater stages VI and VII) is considerably greater than some models predict.

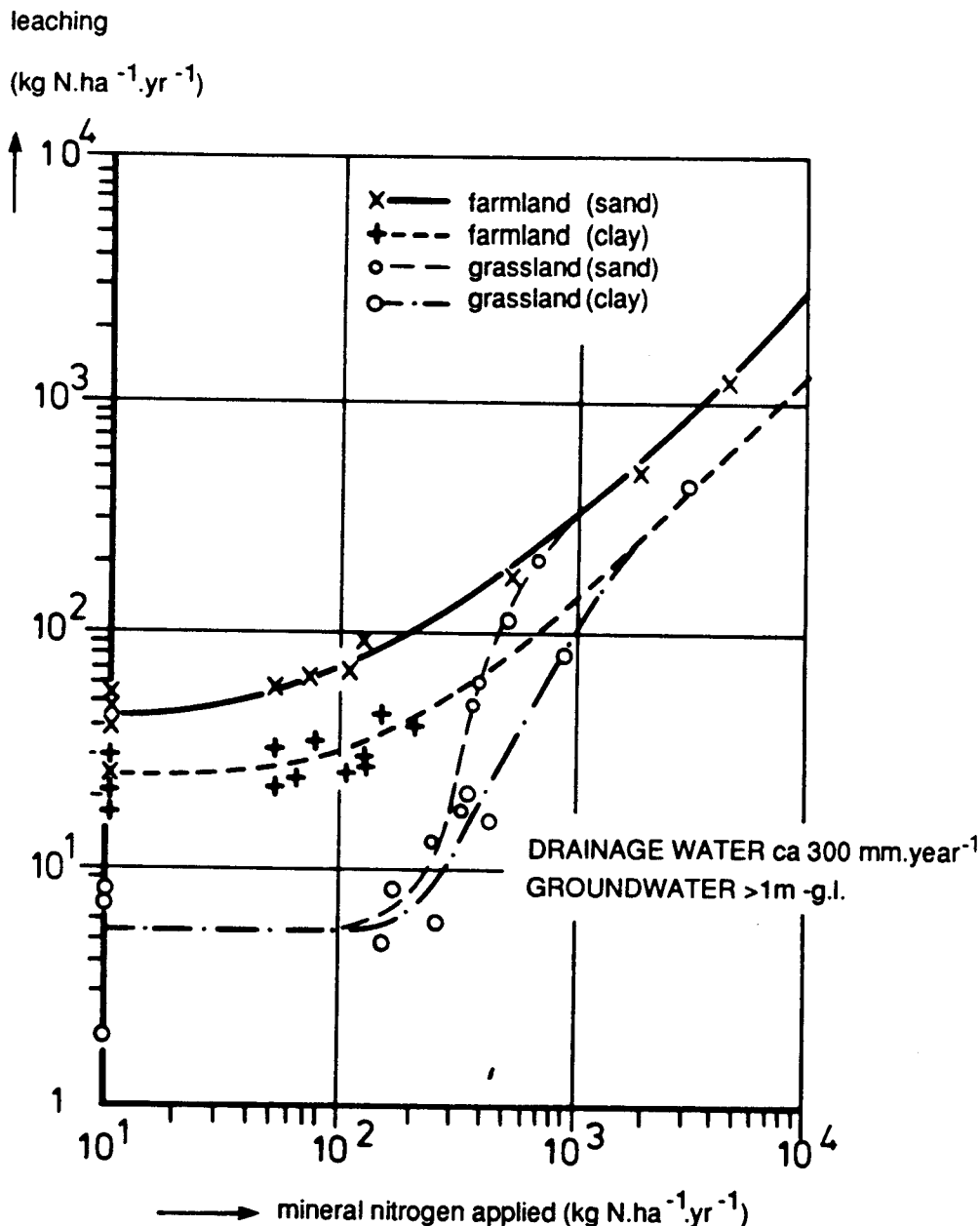


Figure 3.1. Leaching of nitrogen from grass- and farmland

Models which describe the leaching of nitrate into groundwater include the NPK balance model (RIVM), Nitrag (Institute for Soil Fertility), Renlem (Stiboka), WML (WL/DBW-RIZA), Sesoil (EPA), CREAMS (USDA), ANIMO (ICW) and RPC model overfertilization (RIVM).

A simple approach to the calculation of the loading of soil and surface water with nitrate has been presented by the Working Group on Water, Soil and Air (1982). The parts of the hydrological cycle relevant to this are considered in these predictive methods. The method consists of various generalized research results and hence gives an approximation of the nitrate load.

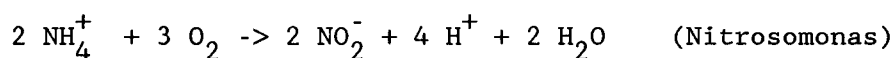
3.1.2. Transformation

Bacteria, partly in symbiosis with plants (especially leguminous plants), convert atmospheric N_2 into organically bound nitrogen.

Organic nitrogen is converted in the soil by mineralization into anorganic products, ammonium and nitrate. Constituent processes of mineralization are ammonification and nitrification. Ammonification is the conversion of organic nitrogen to ammonium.

Nitrification is the microbial conversion of ammonium to nitrate and nitrite. For nitrification to occur, sufficient oxygen must be present.

The chemical equations are:



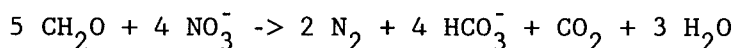
Nitrate that has been converted into other compounds via denitrification no longer contributes to uptake by plants and the contamination of soil and groundwater.

Denitrification is the reduction of nitrate to nitrite, nitrogen gas and other gaseous N compounds. Reduction of nitrite to ammonia or nitrous oxide can also occur. The process can take place via chemical and microbial conversion. Chemical denitrification can occur in the presence of Fe_2^+ and Mn_2^+ . Denitrification by microorganisms is most common and is brought about by facultative anaerobic bacteria under anaerobic conditions.

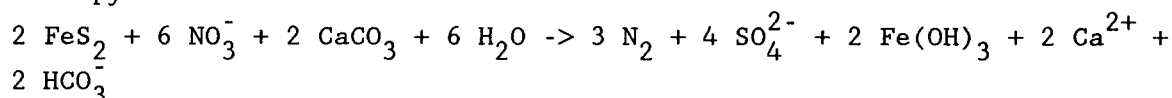
Both organic substances and reduced anorganic compounds (e.g. pyrite) can serve as H^+ donors (Kolle et al., 1983; Van Bennekom, 1982).

The chemical equations are:

With organic matter



With pyrite



It is clear that the hardness is increased in each case. In the case of pyrite oxidation, the sulphate content often rises considerably.

Nitrification and denitrification are processes which take place in both soil and surface water.

Apart from the presence of a substrate and microorganisms, the above-mentioned processes are influenced by temperature, pH, moisture content, the extent to which anaerobic processes can occur, and the nutrient concentrations. Nitrification in the soil becomes negligible below 5 °C. The optimum temperature for this process is about 30 °C; ammonification occurs chiefly between 40 and 60 °C (for example, in a compost heap). Ammonification can also take place in the soil, albeit at a much slower rate than nitrification.

The supply of organic matter to the root zone is in many cases in equilibrium with its decomposition so that, on a yearly basis, the amount of organic matter supplied is also mineralized again.

In soils having a neutral pH, all the ammonium present is converted to nitrate within a few weeks when the temperature is favourable. When the pH of the soil is above 7, nitrogen can vaporize in the form of ammonia.

Nitrification proceeds very rapidly in slightly acidic to alkaline soils (pH 5-8) and slowly in acidic soils (pH 3-5). Nevertheless, high nitrate concentrations are also frequently found in acidic soils (CCRX, 1987). Denitrification, too, proceeds at a lower rate at low pH values (see table 3.5.; CCRX, 1987). Moreover, nitrification leads to acidification of the soil and groundwater.

Denitrification, however, depends chiefly on the extent to which anaerobic processes can occur, which in the water-unsaturated zone is related to the moisture content and thereby also to the groundwater level. Because of the heterogeneity of the soil, nitrification in areas with oxygen, and denitrification in areas without oxygen can occur side by side. In the saturated zone, in the presence of oxidizable compounds, the oxygen present in the groundwater is first used up for oxidation. Only then will nitrate begin to play a role in oxidation reactions brought about through denitrification.

Since the water solubility of nitrate is many times greater than that of oxygen, oxidation of oxidizable compounds, such as organic matter and pyrite, through denitrification in the saturated zone can be more important than oxidation by oxygen.

However, not enough is known about the occurrence of reduced compounds at greater depths which can be oxidized by nitrate.

Table 3.5. Annual fall in the nitrate level of the groundwater (g N.m^{-3}), with a residence time of 1 year in a layer of soil containing either 0.2% or 0.4% organic matter, for several pH values

Organic matter	pH = 6	pH = 5	pH = 4
0.2%	12.2	6.5	0.5
0.4%	25	13	1.0

Knowledge about the occurrence of denitrification in the water-unsaturated zone is related to the depth of the water table below ground level: the groundwater stage (Steenvoorden, 1983).

Knowledge about the occurrence of denitrification in the water-saturated groundwater system is limited. Field studies indicate that denitrification can change the chemical composition of groundwater. However, quantification is hampered by the fact that changes in quality can also be brought about by mixing with other groundwater (CCR, 1987).

3.2. BEHAVIOUR IN SURFACE WATER

3.2.1. Distribution

Because nitrate is readily soluble in water, the transport of nitrate in water is chiefly determined by the transport of water itself. The reader is referred to chapter 2 for the origin of the nitrogen compounds present in surface water.

3.2.2. Transformation

Nitrification and denitrification are processes which take place in the surface water as well as in the soil and soil sediments (see section 3.1.2.). Nitrification requires oxygen, and therefore oxygen deficiency can develop under certain circumstances.

In surface water, the nitrification reaction steps generally occur simultaneously although the first step ($2 \text{NH}_4^+ + 3 \text{O}_2 \rightarrow 2 \text{NO}_2^- + 4 \text{H}^+ + 2 \text{H}_2\text{O}$) proceeds at a faster rate especially in brackish waters, so that nitrite can accumulate under certain conditions. In the marine environment, the density of nitrifying bacteria in saline waters is very low (CCR, 1987) so that nitrification is negligible. In the upper layer of the

sediment, on the other hand, nitrification does take place in both freshwater and salt water. In estuaries or, more generally, in brackish water, the situation is more complex owing to the presence of both freshwater and salt water and the freshwater and marine nitrification that can be distinguished here. The freshwater nitrification products are adsorbed onto suspended material carried by rivers. A study performed in the Scheldt estuary (Van der Kooy, 1982) showed that there was extensive nitrification in the section between Antwerp and Lamswaarde, which decreased in the Westerschelde and was virtually nil near Flushing. Nitrifying bacteria are very sensitive to the chemico-physical conditions of the water.

For example, the temperature is an important environmental factor. Nitrification becomes negligible below 5 °C; the optimum temperature seems to lie around 30 °C. The redox potential (Eh) and acidity (pH) are also important factors in determining the course of nitrification. Nitrification is an important process in surface water as well as in the treatment of waste water because the oxygen concentration is at issue. Allowance should be made for this when discharging waste water. This can be done by letting the nitrification process take place in the purification plant instead of in the surface water. Factors which influence nitrification are: sludge load, sludge age, oxygen concentration, temperature, pH and the presence of inhibitors. The realization of nitrification-promoting conditions can lead to a decrease in the oxygen requirement of the waste water to be discharged.

Nitrate is removed from water mainly through uptake of nitrate by plants (nutrient). In addition, it can be removed by denitrification in soil sediments (Van Kessel, 1977).

The denitrification of nitrate to N_2 and N_2O via the microbial or chemical pathway occurs only under anaerobic conditions. In the case of microbial breakdown, nitrate functions as an electron acceptor in the process of respiration. An organic compound, and occasionally H_2 or a sulphide, can act as the electron donor.

Denitrification takes place over a broad pH range, but decreases with falling pH values (see 3.1.2.). Nitrification can also lead to acidification of surface water.

3.3. BEHAVIOUR IN AIR

3.3.1. Distribution

It is possible to estimate the contributions from area sources and source categories to the concentration and deposition of nitrate from calculations performed with the aid of dispersion models for air pollution. Calculations made by the RIVM using a long-term grid model for NO_x (Van Egmond and Kesseboom, 1985; De Leeuw et al., 1985) put the contribution from NO_x sources in the Netherlands to the concentration of nitrate and nitric acid at 11%. An earlier estimation by the TNO arrived at 12-15% (Duym and Huygen, 1984). The contribution to the wet deposition is small (about 10%), that to the dry deposition relatively large (40-45%). Given the good agreement with measured concentrations and depositions, these calculations can be regarded as reasonably reliable. Because of the improved description of the chemistry of nitrate formation, the discrepancy observed by De Leeuw et al. (1985) between measured and calculated concentrations and wet depositions of nitrate in winter no longer exists in the current version of the model. Estimates from earlier model studies of contributions from foreign area sources have been summarized in table 3.6. (Asman et al., 1984; Duym and Huygen, 1984).

Table 3.6. Contributions of NO_x emissions originating in several countries to the total deposition of NO_x and subsequent products in the Netherlands

Country	Contribution (%)	
	Asman et al. (1984)	Duym and Huygen (1984)
The Netherlands	35	33
Belgium	11	15
Federal Republic of Germany	29	28
France	5	7
Britain	13	9
Czechoslovakia, Hungary	4	1
GDR, Poland	4	2
Denmark, Norway, Sweden	0	2
Others	3	3

Duym and Van Aalst (1984) calculated the contribution of Dutch NO_x emissions, divided into source type, to the deposition of nitrate on areas susceptible to acidification. As table 3.7. shows the largest contributions

are made by traffic, space heating and electricity power stations. Figure 3.2. gives an impression of the variation in this load over the Netherlands (RIVM, 1987).

Table 3.7. Contributions from various source categories to the nitrate and NO_x deposition on vulnerable nature reserves, as a percentage of the contribution from NO_x sources in the Netherlands

Source category	Contribution (%)
Power stations	9
Oil refineries	2
Chemicals industry	6
Metal industry	1
Other industries	3
Local traffic	29
Through traffic	33
Space heating	8
Others	10

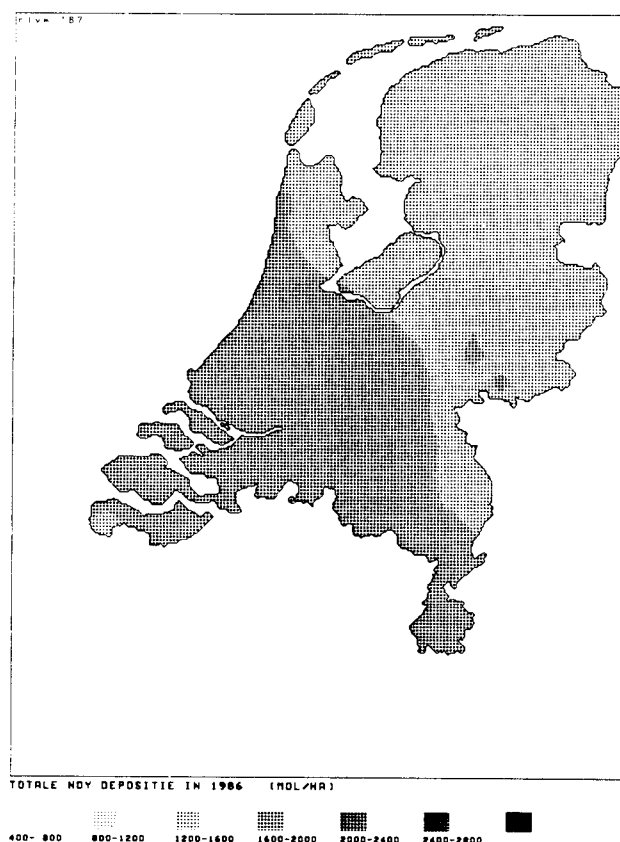


Figure 3.2. Total deposition of NO_y and subsequent products on the Netherlands in 1986 (mol per ha per year) (Erisman et al., 1987); 1 mol per ha per year = 0.014 kg per ha per year

3.3.2. Transformation

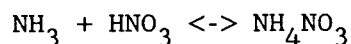
Nitrate is present in the air in various forms:

- as gaseous nitric acid
- as (anorganic) nitrates, in the form of solid or liquid aerosol (particles suspended in the air)
- as organic nitrates, in both gaseous and aerosol form
- as gaseous nitrate radical

These forms behave differently in air (Van Aalst and Diederer, 1985). The most common forms are the (anorganic) nitrates and nitric acid (see tables 4.11. and 4.12.).

Gaseous nitric acid is produced, for example, by reaction of NO_2 with OH radicals. This formation takes place almost exclusively during the day and is important especially in summer; the conversion rate of NO_2 can amount to 20-25% per hour at the high OH concentrations then occurring. As is known, NO_2 is formed from NO (the form in which NO_x is chiefly emitted) by reaction with ozone or with peroxyradicals, which arise when hydrocarbons are oxidized photochemically.

Nitric acid reacts with basic components, especially ammonia, to nitrates:



This conversion is reversible; the position of the equilibrium is strongly dependent on the temperature and the relative humidity; a drop in temperature and increased humidity promote the formation of ammonium nitrate (Harrison and Piao, 1983). As a result of this reaction, a (lowering) effect of emissions of ammonia on the concentrations of nitric acid can be expected.

Nitric acid is rapidly removed through wet and dry deposition (Van Aalst and Diederer, 1985); the deposition velocity is high, possibly about 3.5 cm.s^{-1} (Erisman et al., 1987) which, as an annual average, is equivalent to a time constant of about 35% per hour for removal through dry deposition. The time constant for removal through wet deposition is 1.4×10^{-4} per second, or 36-144% per hour, during rainfall; the concentration will thus fall sharply during a shower. Taking into account the average duration of wet and dry periods (De Leeuw et al., 1985), the annual average time

constant for removal through wet deposition is about 2% per hour. This makes the overall average lifetime of gaseous nitric acid around 10 hours. Nitrate in aerosol is formed by uptake of nitric acid into (alkaline) aerosol or through reaction of ammonia with nitric acid. Nitrate aerosol can also arise via the nitrate radical NO_3 . This can - especially at night - react with NO_2 to form N_2O_5 and this is converted in atmospheric water into nitrate. Uptake of NO_3 into water drops can also lead directly to formation of nitrate aerosol.

Ammonium nitrate and other volatile nitrates decompose at increased temperature in the outdoor air. Little is known about further reactions; nitrate is generally thought to be a stable end-product. Wet deposition is probably the most efficient removal process, with an estimated time constant of 10^{-4} per second during rainfall; averaged over the year, the time constant for wet removal in the Netherlands is 1-2% per hour. The dry deposition velocity is possibly $0.1\text{-}0.4 \text{ cm.s}^{-1}$; great uncertainty exists about this (Van Aalst and Diederer, 1985). The lifetime of nitrate at moderate temperatures, when vaporization does not play a role, is on average 1.5 to 2 days. An important property of nitrate aerosol is its hygroscopic nature. Many nitrates already deliquesce at moderate relative humidity (60-70%) and swell considerably with increasing relative humidity. On the basis of visibility measurements and simultaneous measurements of the aerosol composition, it was estimated (Van Aalst et al., 1983) that the nitrate contribution to the reduction of visibility in the Netherlands as a result of air pollution is about 35%. At normally occurring aerosol concentrations the 50-percentile value of visibility falls from 40 to 14 km when the relative humidity is low (<75%). With high relative humidity (90-98%), visibility is reduced from 10 km to about 2 km.

The nitrate radical is produced when NO_2 reacts with O_3 . Since it is unstable in the light it is observed in measurable concentrations (up to several tenths of $\mu\text{g.m}^{-3}$) only at night (Platt et al., 1984). This is probably how nitrate in aerosol form arises at night. The nitrate radical reacts very quickly, for example, with unsaturated hydrocarbons such as terpenes, derived from emissions by trees and plants (Atkinson et al., 1984) and from the anthropogenic emission of hydrocarbons.

Organic nitrates are produced in this process. Nitric acid is produced when NO_3 reacts with aldehydes (Atkinson and Lloyd, 1984).

Organic nitrates arise, for example, when nitrate radicals (NO_3) react with unsaturated hydrocarbons. Reaction also occurs with saturated hydrocarbons, albeit at a lower reaction rate. Little is known about the properties of these compounds and about their occurrence in the outdoor air. The peroxyacylnitrates constitute a special class. The best known of these is peroxyacetylnitrate (PAN), the concentrations of which are reasonably well known in the Netherlands (Van Aalst et al., 1983). This is to a lesser extent also true of peroxypropionynitrate (PPN) and the strongly tear-inducing peroxybenzoylnitrate (PBzN). These substances arise in the photochemical decomposition of hydrocarbons and aldehydes through reactions of intermediate products with NO_2 . The stability of these compounds in the outdoor air is heavily dependent on the temperature. At 25 °C, the lifetime of PAN is about one-and-a-half hours because of decomposition, and about two days at 5 °C. The dry deposition velocity of PAN above land surfaces is about 0.3 (0.1-0.6) cm.s^{-1} , equivalent to a time constant for removal through dry deposition of around 1% per hour. Very little dry deposition occurs above water. PAN is probably not removed efficiently through precipitation (Van Aalst and Diederer, 1985).

3.4. BEHAVIOUR IN BIOTA

Nitrogen is very important for the growth and structure of crops. Too low an availability leads to poor growth and reduced yields. The nitrogen in the soil available to the plant can be taken up in both the nitrate and the ammonium form. In view of the desired electron neutrality, the plant prefers taking up the nitrogen, along with the important cations, in the negative nitrate form rather than in the positive ammonium form. Part of this is converted in the roots via nitrite and ammonium to amino acids. The remaining nitrate is transported via the vascular bundles to particular sites in the leaf.

Under the influence of two enzymes, viz. nitrate reductase and nitrite reductase, conversion of nitrate to ammonium can here also take place. Since the conversion of nitrite to ammonium proceeds faster than that of nitrate to nitrite, nitrite cannot accumulate in a living plant. The nitrate content of the plant is then determined by the supply of nitrate (uptake and transport) and its conversion (reduction of nitrate in the leaf).

Differences in nitrate content between the various crops are for a large part caused by morphological characteristics. In general, the nitrate content is highest in stems and leafstalks and decreases in the following order: roots, leaf blade, fruit/seed and flower parts. Older plant parts are often richer in nitrate than younger parts. Apart from these morphologically determined differences, nitrate contents vary as a result of differences in species and varieties.

Light is a very important factor in the accumulation of nitrate, because the energy for nitrate reduction is supplied by photosynthesis. Both the light intensity and the length of the light period are important. The well-known fact that higher nitrate levels are generally found in autumn and winter than in spring and summer is largely caused by the light factor. Moreover, vegetables are grown in greenhouses in the autumn and winter, which further reduces the amount of light.

The temperature has a relatively stronger influence on nitrate uptake than on nitrate reduction so that the nitrate content of the plant rises with increasing temperature. The temperature influences the uptake of nitrate indirectly, because the nitrification of nitrogen in the soil is stimulated at higher temperatures, resulting in an increased nitrate supply to the plant (Corré and Breimer, 1979).

3.5. SUMMARY AND CONCLUSIONS

Nitrate is transported in the soil via the soil water and the groundwater. Various models are available for describing the transport of nitrate in the subsoil. However, these models have generally not been validated for the field situation. The extent of denitrification is heavily dependent on numerous factors. This makes choosing an appropriate denitrification factor difficult.

Some insight exists into the relation between denitrification in the unsaturated zone and the groundwater level. The relation to soil type has not yet been studied, and little is also known about denitrification in the saturated zone. The acquisition of more knowledge in this field is necessary for drawing up diversified regulations in a justifiable manner. Owing to different reaction rates, nitrite can accumulate during nitrification, especially in surface water. Although nitrification often predominates in surface water, denitrification in, for example, soil

sediments can also occur. Nitrifying bacteria are very sensitive to the physicochemical conditions of the surface water.

Distribution models are available for the environmental compartment air which describe the concentration and deposition of nitrate in relation to the source areas. The highest air depositions in the Netherlands are found in a zone running from Gelderland (Ruhr area) via Utrecht to South Holland. It generally concerns NO which is converted via NO₂ to nitrate in the atmosphere. Little is known about the occurrence of organic nitrates in the outdoor air. Wet deposition seems to be the most effective process for removing nitrate from the atmosphere.

Plants extract nitrogen from the soil in the form of ammonium or nitrate. In view of the greater solubility of nitrate, nitrate uptake predominates. Part of the nitrate is converted to amino acids in the roots. The remaining nitrate is transported to the leaves and here converted into ammonium. The nitrate content in the leaf is determined by the ratio between the supply of nitrate and its conversion to ammonium. Important factors here are light and temperature. Insufficient insight exists into factors which can inhibit the uptake of nitrate.

4. CONCENTRATIONS IN THE ENVIRONMENT AND EXPOSURE LEVELS

4.1. BACKGROUND CONCENTRATIONS

The element nitrogen is a principal constituent of the atmosphere. The natural nitrogen cycle, thus without being influenced by man, is an essential part of the living environment. Nitrate is formed in this cycle from ammonium (nitrification) which is taken up by plants. Also, free-living and symbiotic organisms fix atmospheric nitrogen. This is the key source of nitrogen supply to plants in nature. Nitrate can be lost from the soil and the surface water by reduction of nitrate to N_2 and N_2O (denitrification). Finally, nitrate can be washed from the land into surface water and it can end up in groundwater by leaching.

As far as is known, natural processes such as forest fires, oxidation of ammonia in the atmosphere and lightning, make only a small contribution to the total emission of the starting compounds for nitrate, these being NO and NO_2 (Veldt, 1983).

The anthropogenic influence (by combustion processes, fertilization and intensive livestock farming) on the nitrogen cycle is considerable. The natural concentration of nitrate in the soil and groundwater depends heavily on the soil type and the hydrological situation (section 4.2.).

The natural concentration of nitrate in rivers varies from 0 to 4 mg NO_3^- -N.l⁻¹. The nitrogen cycle is shown schematically in figure 4.1.

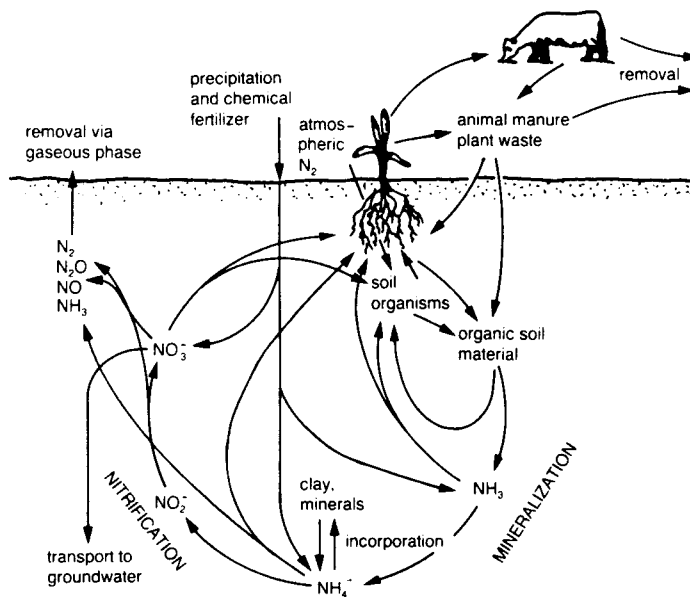


Figure 4.1. Schematic representation of the nitrogen cycle (Buckman and Brady, 1969)

4.2. OCCURRENCE IN SOIL

The nature and occurrence of N compounds in groundwater are determined naturally to a large extent by the structure of the subsoil and the hydrological situation. Soil type and depth of the groundwater level below the surface, in particular, are important factors in this connection. Here, a distinction can be made nationally between areas of marine clay, river clay, peat, sand and loess (CCRX, 1987).

Anaerobic conditions prevail in the freshwater as well as in the brackish and salty groundwater in marine-clay and peat areas. Consequently, oxidized N compounds such as NO_3^- will generally not be found in measurable quantities. In river-clay areas, too, the groundwater is more or less anaerobic, containing very little nitrate or none at all. The surface of the groundwater in sandy areas is not protected by the presence of relatively poorly permeable layers, such as clay or peat layers. Percolating rainwater reaches the water-carrying body directly after passage through the unsaturated soil. Because of the presence of a thick zone not saturated with water (> 2 m) under the high and medium-high sandy soils, aerobic, oxygen-containing groundwater is frequently found up to great depths (up to even 70 to 100 metres below ground level; m -g.l.). This groundwater contains naturally very little nitrate, or none at all. Whether or not nitrate occurs in this groundwater is determined to a large extent by land usage and hence by the fertilizer doses applied and, in the case of nature reserves, by the type of vegetation and atmospheric deposition. Depending on the intensity of fertilization, nitrate concentrations of tens of mg.l^{-1} can be found up to depths of more than 70 metres, and even exceed 100 mg.l^{-1} in the upper groundwater layer. The groundwater level in low-lying sandy soils is quite near the surface of the ground ($< 2\text{m}$ -g.l.). Because of this, the conditions for the presence of anaerobic groundwater are more favourable, so that denitrification occurs. The groundwater in these sandy areas usually does not contain high nitrate concentrations, but it should be noted that it is especially on the high and medium-high sandy soils that intensive livestock farming is practised. The nitrate concentrations decrease with depth, partly because the extent to which anaerobic processes can occur increases with depth, and partly because of the presence of older groundwater here, which has been exposed to air pollution to a lesser extent than more recent, shallow groundwater. Locally, the presence of peat layers in the subsoil leads to

denitrification. Results from the National Groundwater Quality Monitoring Network (Van Duijvenbooden, 1987) show that under sandy soils used as farmland, the average nitrate concentration of the groundwater at 10 m -g.l. ($24 \text{ mg NO}_3^- \cdot \text{N.l}^{-1}$) is considerably higher than the maximum concentration permitted under the Water Board Decree and is also higher than the EEC standard for drinking water (11.3 mg N.l^{-1}). There is a clear rise in the nitrate concentrations in the groundwater at numerous measurement sites (at at least 25% of all measurement points of the National Monitoring Network which are situated in the sandy areas). Over a 6-year period, the average nitrate concentration in the groundwater in agricultural areas also rose from 19 to 24 mg N.l^{-1} (period 1981-1986). The nitrate concentration at 10 cm -g.l. was found to be higher than the drinking water level at about 50% of the measurement sites of the National Monitoring Network in these areas. For the sandy areas as a whole, this is 20% (Van Duijvenbooden, 1987).

The nitrate concentration in groundwater under grassland has more than doubled, from 1.7 to 3.9 mg N.l^{-1} . Besides, recent research (De Wit, 1985, 1987; Boumans, 1987) showed that under grassland with slightly lower water tables (groundwater stages 6 and 7), the shallow groundwater can contain tens of $\text{mg NO}_3^- \cdot \text{N.l}^{-1}$. It is expected that this groundwater containing high nitrate concentrations will gradually migrate downwards, at a rate corresponding to the vertical velocity component of the groundwater. South Limburg differs from the rest of the Netherlands in the presence of loess on chalk. This region is very vulnerable to nitrate contamination because of very low water tables here in conjunction with the rapid transport of groundwater via crevices and holes in the chalk. Despite the relatively low level of fertilization in this region, fairly high nitrate concentrations are nevertheless found in the groundwater. At several groundwater pumping stations in South Limburg, clearly increasing and relatively high nitrate concentrations are consequently found in the pumped raw water.

The nitrate content in forests and nature reserves is naturally very low (0.2 - 0.5 mg N.l^{-1} ; Bots et al., 1978; Steenvoorden, 1983). As a result of vaporization of ammonia on nearby agricultural plots followed by entrapment in the forest areas, the concentrations can however rise sharply and levels as high as several tens of $\text{mg NO}_3^- \cdot \text{N.l}^{-1}$ are found (Krajebrink, 1987).

A summary of the occurrence of nitrate in fresh groundwater for different soil types is given in table 4.1., and in table 4.2. in fresh groundwater in sandy areas related to land use (Van Duijvenbooden et al., 1985, 1987).

Table 4.3. gives this information for shallow groundwater (De Wit, 1985, 1987).

Table 4.1. Occurrence of nitrate in fresh groundwater ($\text{Cl}^- < 200 \text{ mg.l}^{-1}$) for different soil types in mg N.l^{-1}

Soil type	<u>Sampling depth below ground level</u>	
	- 10 m	- 25 m
Sand	5.9	1.4
River clay	1.2	0.4
Marine clay	0.1	0.1
High moor peat	2.1	0.1
Low moor peat	0.1	0.1
Loam		1.1
		0.1

Table 4.2. Average nitrate concentration (in mg N.l^{-1}) in fresh groundwater ($\text{Cl}^- < 200 \text{ mg.l}^{-1}$) in sandy areas with different land use

Land use	<u>Sampling depth below ground level</u>		
	- 10 m	- 15 m	- 25 m
Agricultural land	24	9.9	3.9
Grassland	3.9	0.7	0.4
Nature reserve	1.5	0.6	0.5
Housing	7.4	2.3	2.3
Dunes	0.4	0.1	0.1

Table 4.3. Average nitrate concentrations in the upper layer of the groundwater in mg N.l^{-1} (De Wit, 1987)

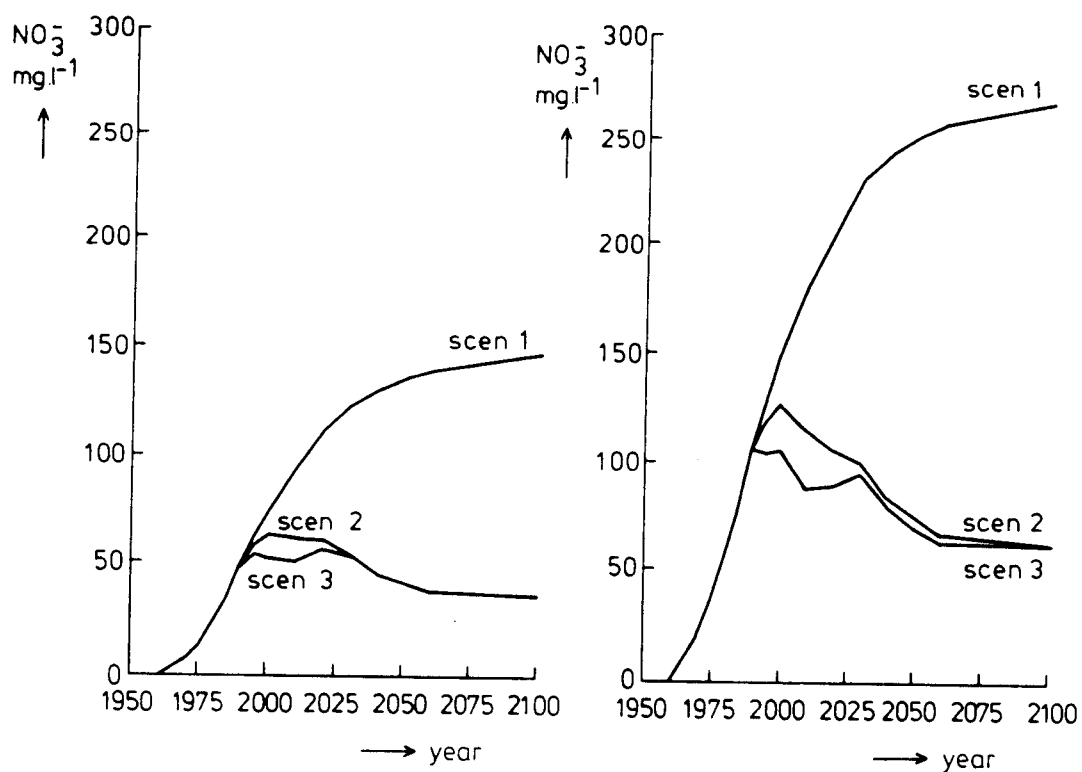
	Clay	Peat	Sand
Agricultural land	21.7	-	62.6
Grassland	3.0	0.6	23.9
Nature	2.9	0.8	2.2

At numerous groundwater collection points in the sandy areas, the nitrate concentrations in the water obtained are gradually increasing (see 4.5.2.). In view of the often very high nitrate concentrations in the shallow groundwater, it may be expected that for the time being this trend will also continue in the future. Any effects of measures to be taken will only become noticeable in the long run. Besides, the concentrations cannot be deduced from the quality of the groundwater in the catchment area without

further details. Within the catchment area of a pumping station, several subregions can often be distinguished with diverse land usage and different soil types. As a result, mutually divergent water qualities can be found in these subregions. Depending on the catchment area, the quality of the groundwater drawn from each borehole can also differ. Various models have been developed which describe the water quality of various environmental scenarios. A practical example of this is shown in figure 4.2. for pumping stations in the Achterhoek (RIVM, 1987).

Land use	
urban buildings	18%
grassland	23%
forest	38%
arable land	10%
maize	11%

Land use	
urban buildings	18%
grassland	61%
forest	0%
arable land	10%
maize	11%



Scenario 1: Continuation of the current level of fertilizing, which implies leaching of nitrate into the groundwater below:

-grassland	325 mg.l^{-1}
-arable land	200 mg.l^{-1}
-maize land	575 mg.l^{-1}

Scenario 2: From 1988: Manure Decree

The effect of the Manure Decree on nitrate leaching:

	grassland	arable land	maize land
1988-1991	251	121	401
1992-1995	179	121	243
1996-2000	143	121	204
2001-final phase	69	67	87

Scenario 3: Idem 2, with the understanding that in the 25-year protection zone of a pumping station the Decree's ultimate standard takes effect immediately.

Figure 4.2. Future nitrate content in the raw water of two pumping stations (examples) in the Achterhoek. For different fertilization scenarios (Source: RIVM)

4.3. OCCURRENCE IN SURFACE WATER

4.3.1. State-owned waters

One of the nitrogen compounds in the surface water is nitrate. The principal sources of nitrate in the Netherlands are: household waste water, washout from air and soil by rain, and industries. The increase in the nitrate load of the surface water since the beginning of this century is mainly caused by:

- population growth (more connections to the sewerage system and more industrial activities);
- increased fertilization in agriculture;
- an increase in the transboundary load.

The last-mentioned cause is generally the largest source of N compounds in our surface water. As a result of the growth in the number of purification plants since the seventies, the loading with nitrate became relatively greater whereas that with organic N compounds and NH_4^+ decreased, because of nitrification (aerobic conversion of N compounds into nitrate). The amounts of nitrate entering our country via the Rhine and the Maas over the past 5 years are given in table 4.4.

Table 4.4. NO_3^- load of the Rhine and the Maas ($\text{kg NO}_3^- \cdot \text{s}^{-1}$)

Year	Rhine/Lobith	Maas/Eysden
1980	39.6	3.4
1981	49.1	4.2
1982	54.6	3.2
1983	42.8	3.4
1984	44.9	4.2

(Quality survey of state-owned water bodies)

The nitrate concentrations in the large rivers have stabilized in recent years (see figure 4.3. and table 4.5.).

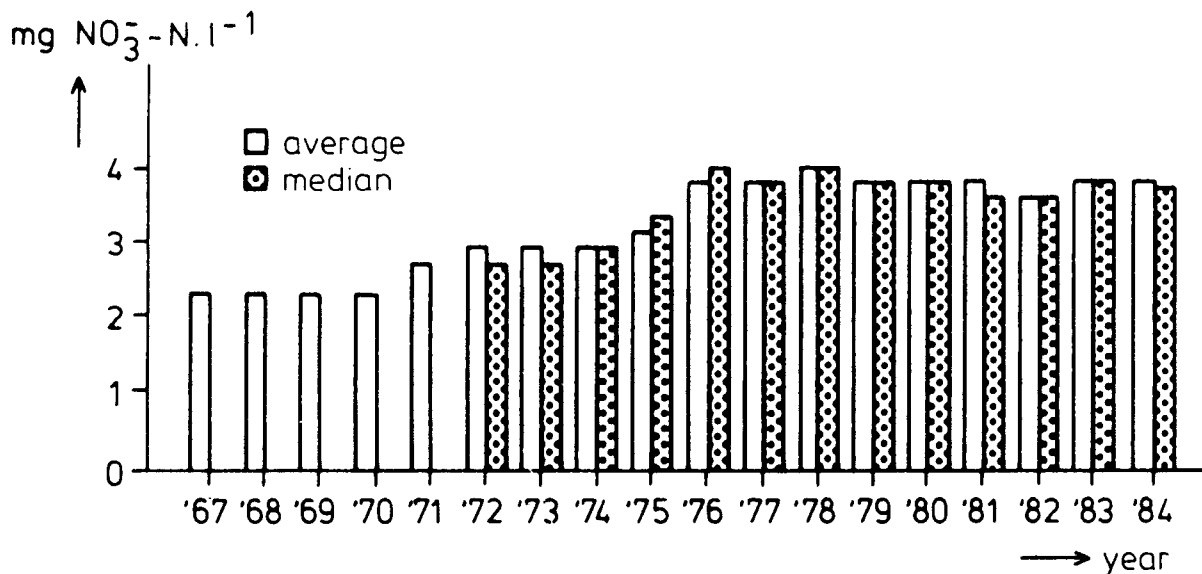


Figure 4.3. Annual averages and medians of the nitrate concentration in the Rhine at Lobith (up to 1983 inclusive, taken from the Composition of the Rhine Water 1982 and 1983)

Table 4.5. Nitrate concentration (annual average in mg NO₃-N.l⁻¹) in the Maas at Eysden (RIZA, 1985).

Year	NO ₃ ⁻ -conc. (mg NO ₃ ⁻ -N.l ⁻¹)
1980	2.8
1981	2.6
1982	2.5
1983	2.6
1984	2.8

In 1984 the annual average nitrate content of the Rhine at Lobith was 3.9 mg NO₃⁻-N.l⁻¹. The nitrate concentrations in the Rhine and the Maas fluctuate widely with the season (see figure 4.4.).

Factors playing a role here are:

- temperature-dependent nitrification;
 - change in the flow in connection with melt- and rainwater;
 - less leaching and runoff of NO₃⁻ in the growing season;
- These factors lead to a lower NO₃⁻ concentration in summer.

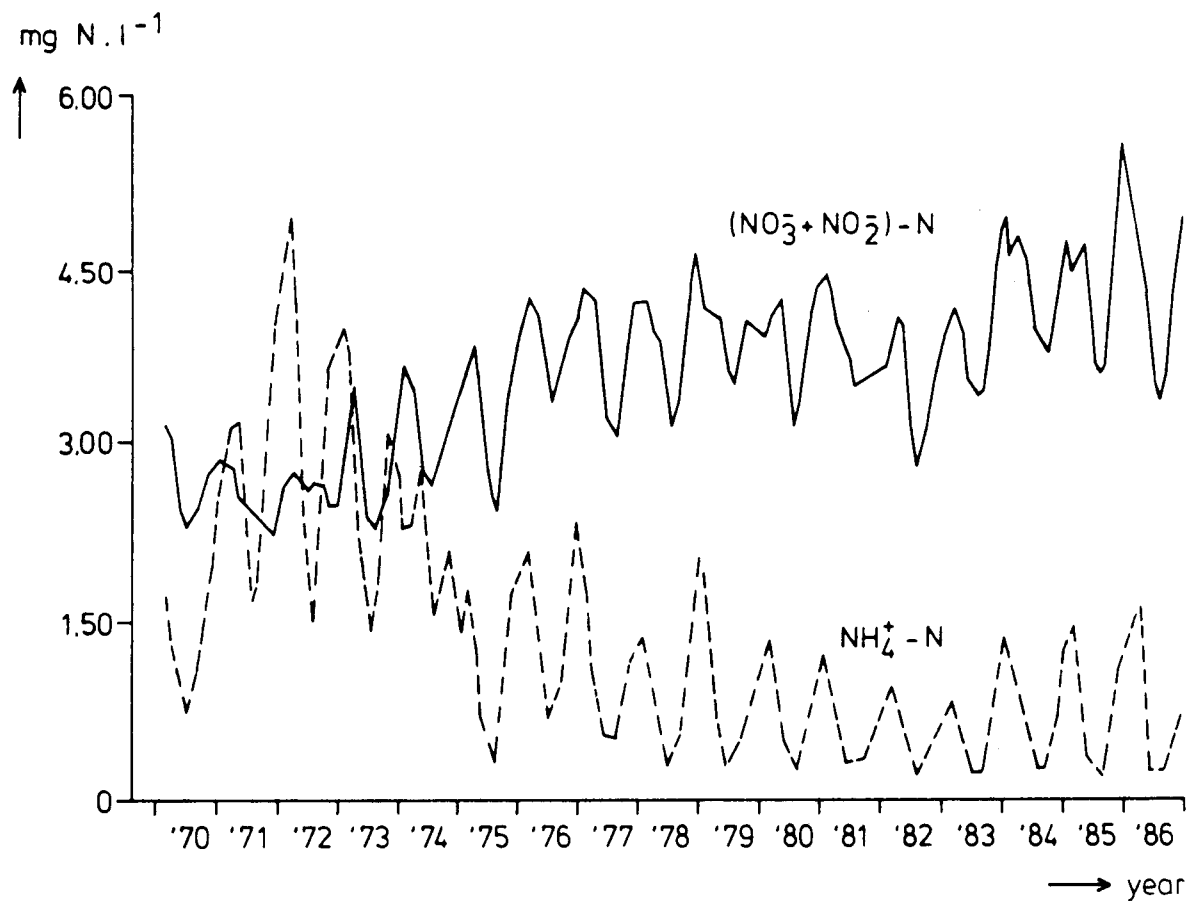


Figure 4.4. Three-monthly average contents of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ in the Rhine at Lobith (Dijkzeul, 1982)

Table 4.6. Annual average of the nitrate concentration ($\text{mg NO}_3^-\text{-N.l}^{-1}$) in the IJsselmeer at Station Y 23
(From: Quality survey in state-owned water bodies).

Year	NO_3^- -conc. ($\text{mg NO}_3^-\text{-N.l}^{-1}$)
1980	2.3
1981	2.3
1982	2.2
1983	2.3
1984	2.8

4.3.2. Non-state water bodies

The NO_3^- concentration in surface water can vary widely because of differences in land use, soil type and (geo)hydrological situation. An example of this is given in table 4.7. (Bots et al., 1978).

Table 4.7. Influence of the soil type and type of surface water on the content of nitrate ($\text{mg NO}_3^- \cdot \text{N.l}^{-1}$) in the summer and winter periods in the provinces of Groningen, Friesland, Drente and part of Overijssel

Soil type	<u>NO₃- concentration</u>		Surface water
	summer ($\text{mg NO}_3^- \cdot \text{N.l}^{-1}$)	winter ($\text{mg NO}_3^- \cdot \text{N.l}^{-1}$)	
Clay	0.1	2.6	ditches
	0.1	1.5	pools
	0.5	2.3	polder drainage ("boezem") water
Clay on peat	0.2	3.1	ditches
	0.2	1.4	pools
	0.3	2.0	polder drainage ("boezem") water
	0.4	-	lakes
Low moor peat	0.4	2.3	ditches
	0.1	0.9	peat pots, pools
Sand	0.2	5.0	streams

The nitrate concentration in summer is significantly lower than in winter as a result of enhanced biological processes and longer residence time of the water.

Higher concentrations are found in streams in sandy areas where there is intensive livestock farming (e.g. on the Veluwe). Values of $8.4 \text{ mg NO}_3^- \cdot \text{N.l}^{-1}$ have been found in the Peel region (Steenvoorden, 1983).

4.3.3. The North Sea

The nitrate concentration in the North Sea is partly determined by the discharge of the large rivers (see table 4.8.) and partly by atmospheric deposition and mineralization of organic compounds. Two decreasing concentration gradients can be distinguished: one in a northerly direction and one perpendicular to the coast. The decrease in the concentrations in the gradient directions mentioned is due to dilution by mixing with water from the Channel. As a result of accelerated processes and a reduced supply, lower nitrate contents are observed in summer than in winter (see figures 4.5. and 4.6.).

The concentrations measured at the different sampling sites are a factor of 5 to 10 lower than in the rivers (figure 4.5.).

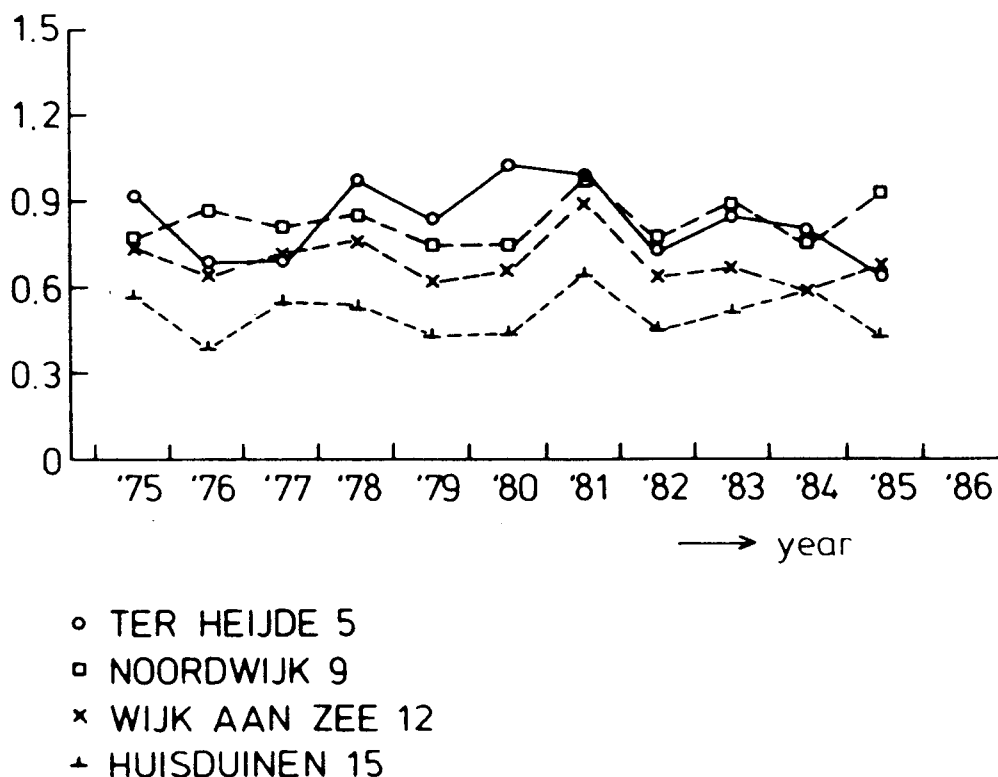


Figure 4.5. Annual averages of nitrate and nitrite concentrations along the North Sea coast (mg.l^{-1}) (CCR, 1987)

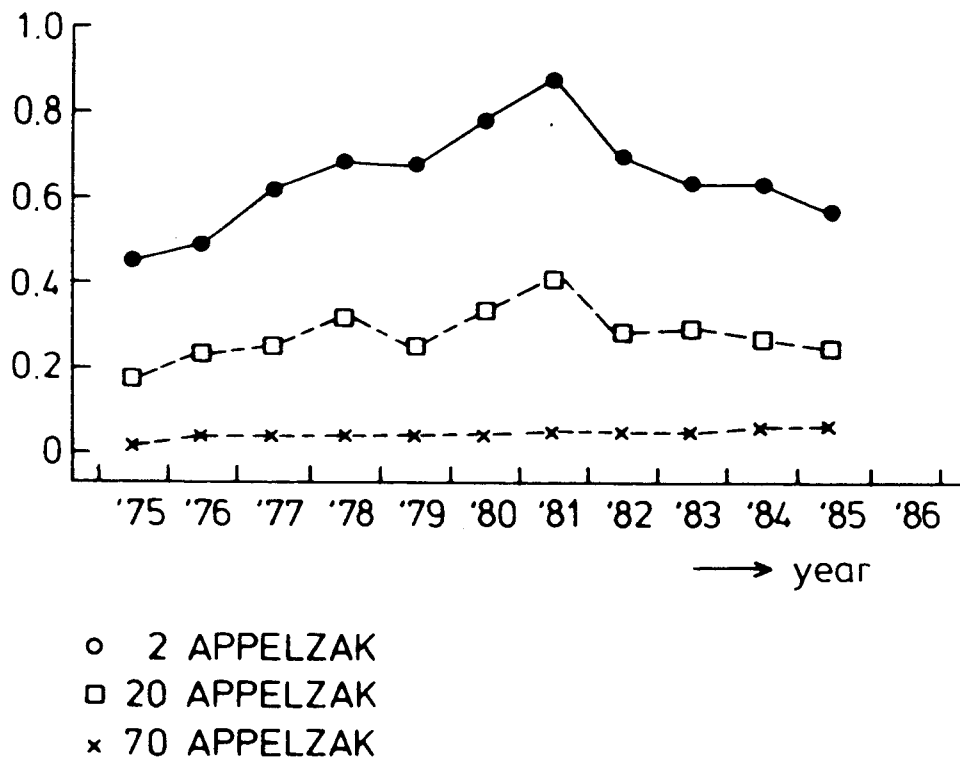


Figure 4.6. Summary of nitrate + nitrite concentrations in the North Sea from east to west, in mg.l^{-1} (annual average)

4.3.4. The Wadden Sea

The nitrate content of the Wadden Sea has risen in the past 10 years (figure 4.7.). The West Wadden Sea is made up of around 15 % Rhine water (CCR_X, 1987). The nitrate content exhibits a distinct seasonal course (figure 4.7.). This is probably related to the reduced influx of freshwater from the IJsselmeer in the summer and to uptake by algae during this period (CCR_X, 1987).

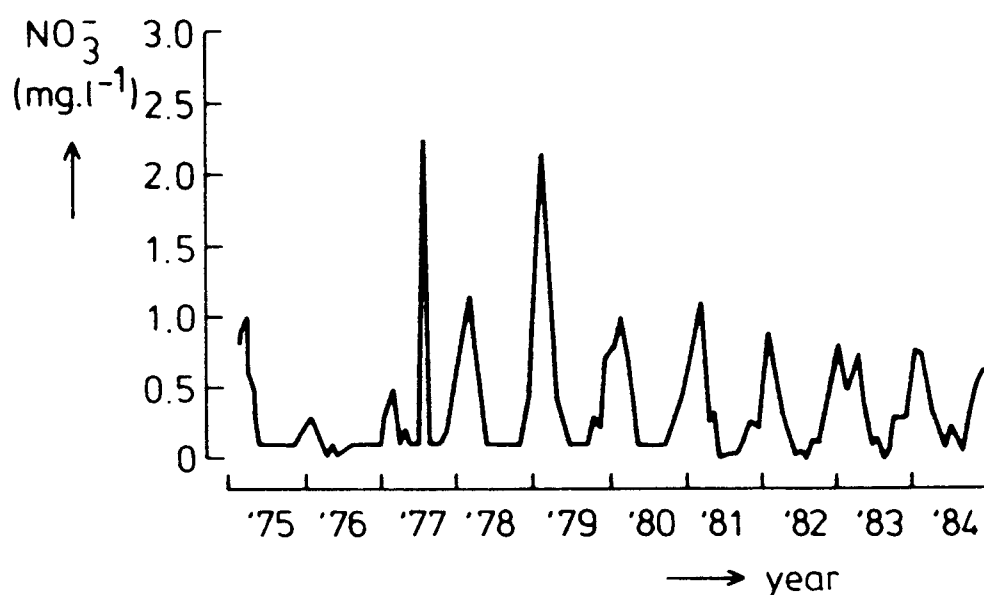


Figure 4.7. Annual average nitrate concentration in the Wadden Sea (Vlietstroom) (mg.l⁻¹)

Table 4.8. Summary of the nitrate load of the North Sea (in 1000 tonnes per year) discharged by various watercourses. Averaged over the period 1979-1981 (CCRX)

	Discharge (m ³ .s ⁻¹)	NO_3^- -N (1000 tonnes per year)
Nieuwe Waterweg	1,463	177.4
Haringvliet	1,145	134.6
North Sea canal	115	8.2
IJsselmeer	504	37.2
Westerschelde	127	23.8
Eems-Dollard	-	8.8
Old Rhine	8	0.7

4.4. OCCURRENCE IN AIR

4.4.1. Indoor air

Little is known about indoor air pollution by nitrate aerosol. American research showed a relation between indoor and outdoor concentrations, as given in table 4.9.

Table 4.9. *Ratio of indoor to outdoor concentrations for nitrate aerosol*

<i>Place</i>	<i>Ratio</i>	<i>Reference</i>
<i>Dwellings with gas cookers</i>	<i>3.0</i>	<i>Yocom (1982)</i>
<i>Dwellings with electric cookers</i>	<i>0.6-1(1)</i>	<i>Yocom (1982)</i>
<i>Offices</i>	<i>0.43</i>	<i>Yocom (1982)</i>
<i>Telephone offices</i>	<i>0.01-0.65</i>	<i>Sinclair et al. (1985)</i>

(1) 2.73 in one case

Gas cookers seem to be a source of nitrate which, considering its important contribution to NO₂ concentrations indoors, is not surprising. In dwellings without nitrate sources the ratio between indoor and outdoor concentrations is less than one. It is not entirely clear why higher ratios are occasionally found in dwellings with electric cookers. There is interest in indoor air concentrations on account of corrosion of electric circuits (Yocom, 1982).

4.4.2. Outdoor air

The concentrations of nitrate in aerosol as measured at various stations in the Netherlands are summarized in table 4.10.

Table 4.10. Concentrations of nitrate in aerosol ($\mu\text{g} \cdot \text{m}^{-3}$), based on 24-hour measurements (RIVM, 1986; Diederer, 1984; KEMA, 1982)

Station	Year	Average	Percentiles			Maximum
			50	95	98	
Delft	1979	6	4	16	21	54
Delft	1980	8	6	22	27	38
Delft	1981	8	6	23	30	54
Terschelling	1981	5	4	17	22	39
Vlaardingen	1981	8	5	24	31	64
Vlaardingen	1984	8	5	22	28	34
Bilthoven	1984	8	6	20	25	49
Biddinghuizen	1984	7	5	16	20	27
Eindhoven	1981	8	6	23	31	43
Biest-Houtakker	1984	7	5	19	25	31
Vredepeel	1984	8	6	20	22	30
Eibergen	1984	8	6	19	41	118
GAME(1)	1979/1980	6	4	17	24	-

(1) mean of 9 stations, spread across the Netherlands

It shows that the concentrations over the country do not vary much. In Delft the ratio between average summer and winter concentrations in the period 1979-1981 was about 1.2. The nitrate concentrations are raised during pollution episodes in both summer and winter. Therefore, nitrate is probably not derived solely from photochemical conversions. The highest concentrations are however reached in summer. The concentrations are highest with an easterly wind.

The concentrations of nitric acid, based on 24-hour measurements, are given in table 4.11.

Table 4.11. Concentrations of nitric acid, based on 24-hour measurements (in $\mu\text{g.m}^{-3}$; Diederer, 1984).

Station	Year	Average	Percentiles			Maximum
			50	95	98	
Delft	1979	5	4	13	17	23
	1980	4	3	12	17	22
	1981	3	2	13	18	24
Terschelling	1979	4	3	10	15	19
	1980	3	2	9	11	15
	1981	3	2	11	17	20
Vlaardingen	1981	4	3	15	22	32
Eibergen	1981	5	3	17	29	44

Because of the measuring technique used (impregnated filter with prefilter), the concentrations are possibly overestimated, owing to decomposition of nitrate on the prefilter; this error is probably not greater than 25 %. The concentrations of nitric acid likewise exhibit a clear episodic character. Only in summer do sharply increased levels occur during air-pollution episodes. The average concentration in summer is 1.5 to 1.8 times higher than in winter.

4.4.3. Deposition

Wet deposition

The average wet deposition can be calculated from concentration measurements of nitrate in precipitation and from the amount of precipitation as reported by the KNMI/RIVM (RIVM, 1985) over the period 1978-1983. It is given in figure 4.8. Since the measurements were made in open collectors, the values are possibly overestimated owing to dry deposition in the collectors; this error is probably not greater than 30 % (Ridder, 1984).

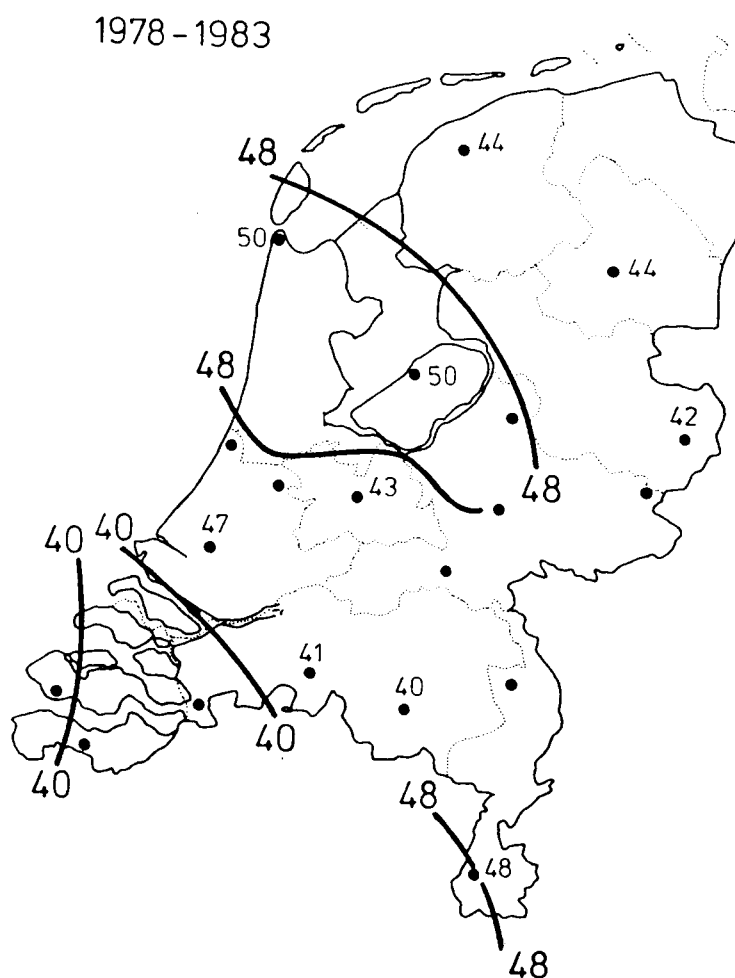


Figure 4.8. Measured wet deposition of nitrate ($\text{mmol.m}^{-2}.\text{year}^{-1}$)

The average wet nitrate deposition on the Netherlands is $470 \text{ mol.ha}^{-1}.\text{year}^{-1}$, or $6.6 \text{ kg N.ha}^{-1}.\text{year}^{-1}$. The wet depositions do not vary much across the country, viz. between 400 and $550 \text{ mol.ha}^{-1}.\text{year}^{-1}$ (5.5 - $7.7 \text{ kg N.ha}^{-1}.\text{year}^{-1}$) (see figure 4.8.). Measurements made by the TNO revealed that the concentration of nitrate in rainwater has more than doubled since 1960 (Guicherit and Van den Hout, 1982), as is shown in figure 4.9.

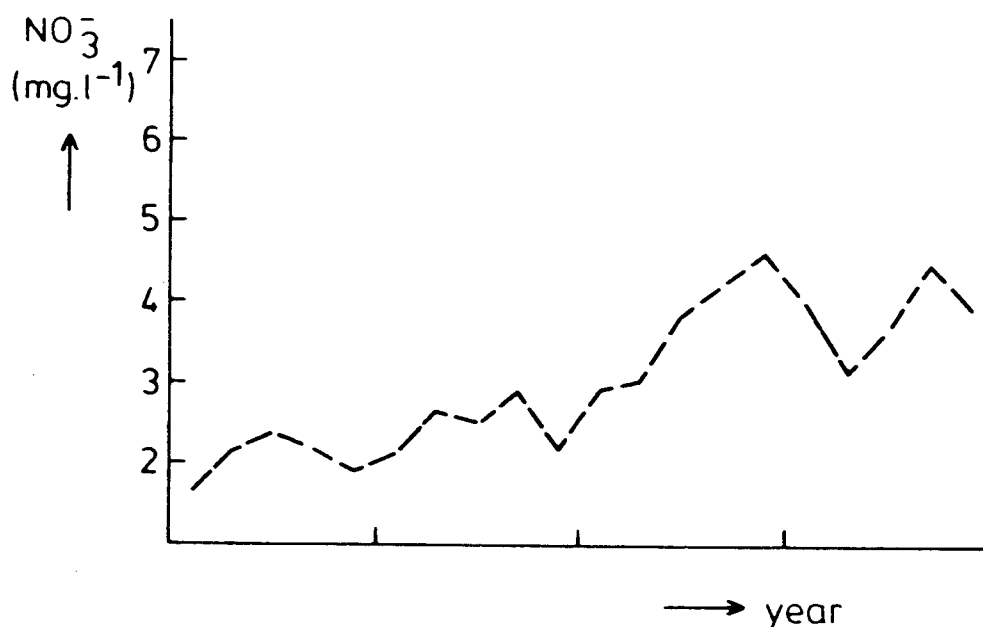


Figure 4.9. Concentrations of nitrate in rainwater during the years 1961-1981, measured in the west of the Netherlands (Guicherit and Van den Hout, 1982)

Dry deposition

No experimental data are available on the dry deposition of nitrate and nitric acid. The deposition can be estimated from average concentrations and deposition velocities (table 4.12.).

Table 4.12. Dry deposition of nitrate

Component	Concentration ($\mu\text{g.m}^{-3}$)	Deposition velocity (cm.s^{-1})	Dry deposition	
			($\text{mol.ha}^{-1}.\text{yr}^{-1}$)	($\text{kg N.ha}^{-1}.\text{yr}^{-1}$)
NO ₃ ⁻	7	0.1 - 0.4	40 - 140	0.5 - 2
HNO ₃	4	ca 2	ca 400	ca 6
Total nitrate			ca 500	ca 7

It shows that the wet and dry depositions of nitrate are about the same. However, dry deposition of NO, NO₂ and PAN also results in input of nitrogen to the soil, namely around 700 mol.ha⁻¹.year⁻¹, or around 10 kg N.ha⁻¹.year⁻¹ (Van Aalst, 1984). The total N input to the soil through dry deposition of NO₃⁻ and subsequent products thus amounts to about 1200 mol.ha⁻¹.year⁻¹, or 17 kg N.ha⁻¹.year⁻¹, which is 2.5 times greater than

the input from wet deposition. This additional contribution to the nitrate input does not apply to water; the deposition velocities of NO, NO_x and PAN above water are negligible. The estimates given here are consistent with very recent calculations (Erisman et al., 1987).

The uncertainty in this estimated dry deposition is considerable, possibly 50 %.

Total deposition

The total deposition of NO_x and subsequent products on the Netherlands is, on average, about 1700 mol.ha⁻¹.year⁻¹ (see also figure 3.2.).

4.5. OCCURRENCE IN FOOD AND DRINKING WATER

4.5.1. Food

An idea of the average daily nitrate intake (table 4.13.) can be deduced from results of analysis of the standard food parcel (CCRX, 1985; Staarink and Hakkenbrak, 1984).

Table 4.13. Division of the daily average nitrate load over the various food groups (calculated as nitrate)

Food group	Consumption (g per day)	Content (mg per kg)	Load (mg per day)
Grain products	160	10	2
Potatoes	160	60	10
Vegetables and legumes	154	see table 4.12.	120
Fruit	154	20	3
Nuts, coffee, tea, cacao	34	-	-
Sugar, "visible" fats	155	-	-
Milk (products)	420	1	-
Cheese	25	8	-
Meat (products)	140	50?	7?
Egg (products)	28	-	-
Fishery products	14	3	-
Beer, wine and spirits	275	5?	1?
Total food			ca 143

? = no Dutch data available; data derived from foreign measurements

The results from 12 Food Inspection Departments, the RIV, RIKILT and CIVO-TNO, obtained during the period 1972 to early 1982, have been processed in table 4.14.

Table 4.14. Nitrate contents in crops

<i>Crop</i>	<i>Highest and lowest medians found (mg NO₃⁻.kg⁻¹)</i>	<i>Crop</i>	<i>Highest and lowest medians found (mg NO₃⁻.kg⁻¹)</i>
Turnip tops	4800	Cauliflower	24-960
Purslane(1)	1600-7200	Carrot	60-900
Spinach	900-5400	Savoy cabbage	110-840
Lettuce	750-5500	Kale	30-530
Lamb's lettuce	3000-3600	French and string beans	300-530
Chinese cabbage	3100	Celeriac	330-490
Parsley	1700-3500	Red cabbage	160-420
Celery	1300-3700	Soup greens	310-320
Endive	730-4000	Cucumber	60-400
Beetroot	1400-3000	White cabbage	100-270
Kohlrabi	2200	Melon	150
Blanched celery	1800	Potato	46-130
Chervil	1600	Paprika	16-160
Oxheart cabbage	590-2600	Tomato	6-160
Radish	780-2400	Strawberry	72-160
Leek	270-2200	Red currant	3- 56
Rhubarb	400-1600	Brussels sprouts	9 -40
		Chicory	30
		Apple	30
		Pear	30
		Asparagus	28

(1) for biodynamically grown products 1600; for other products 4500-7200.

Table 4.13. shows that most of the daily nitrate intake via food is derived from vegetables. This conclusion is substantiated by foreign findings (Trempe, 1981).

When vegetables are cooked, about 40 % of the nitrate is lost with the cooking water, so that the nitrate intake falls in proportion.

The RIVM (Stephany and Schuller, 1980) has analyzed duplicate samples of food consumption per 24 hours. On average, 109 mg nitrate was found with a range of 9-740 mg.

The acceptable daily intake (ADI) was exceeded in 14 % of the samples. In a similar study by the CIVO-TNO made in 1976-1978, involving a smaller number of samples, the range was 36-170 mg.

In 1983 the nitrate contents of biodynamically grown purslane were measured. The nitrate contents in this study amounted to 1800 mg.kg^{-1} , on average, against 6000 mg.kg^{-1} with the conventional cultivation method. Research conducted by the Food Inspectorate of Haarlem in 1972-1974 indicated that in summer nitrate contents in biodynamically grown lettuce and endive are significantly lower than those in the conventionally grown products; such a difference was not found during the winter months. Analysis by the RIVM of 44 samples of ready-to-eat baby and infant food yielded an average nitrate content of $92 \text{ mg NO}_3^-. \text{kg}^{-1}$ (median $45 \text{ mg NO}_3^-. \text{kg}^{-1}$). The highest nitrate content measured was $570 \text{ mg NO}_3^-. \text{kg}^{-1}$. High nitrate contents were found especially in products containing spinach; 5 out of the 6 samples with a nitrate content of more than $200 \text{ mg NO}_3^-. \text{kg}^{-1}$ contained this vegetable.

4.5.2. Drinking water

Exploratory research into the nitrate problem in the Netherlands based on, among other things, the VEWIN (1982) statistics (KIWA, 1984) shows that in that year the nitrate standard of $50 \text{ mg NO}_3^-. \text{l}^{-1}$ ($11.3 \text{ mg NO}_3^- - \text{N.l}^{-1}$) was not exceeded in any of the water-collection areas. One or more wells in the water-collection areas did, however, exceed this standard (only where groundwater was extracted). The EEC recommended value of $25 \text{ mg NO}_3^-. \text{l}^{-1}$ ($5.6 \text{ mg NO}_3^- - \text{N.l}^{-1}$) was exceeded at 8 water supplies. The standard for drinking water is frequently exceeded at private wells. At the request of the Chief Inspector of Public Health for Environmental Protection, the RIVM conducts every year a survey of numerous physical and chemical parameters, including nitrate, at all pumping stations. Although this survey is only a random check (most companies test the nitrate concentration every four weeks), the nitrate concentration was found to agree well with the average values reported by the companies (VEWIN, 1982). The results of this annual survey are given in table 4.15. for 1984 and 1985 (Van Dijk, 1987).

Table 4.15. Classification of the Dutch drinking-water pumping stations (surface- and groundwater) according to the nitrate content of the water supplied in 1984 and 1985

NO_3^- conc. (mg.l^{-1})(1)	Number 1984	Percentage 1984	Number 1985	Percentage 1985	Number 1986	Percentage 1986
< 5	180	69.7	164	68.5	181	71.0
5 - 10	32	12.4	30	12.6	30	11.8
10 - 25	34	13.4	30	12.6	34	13.3
25 - 50	11	4.2	15	6.3	9	3.5
> 50	1	0.3	0	0	1	0.4

(1) To express in $\text{mg NO}_3^- \cdot \text{N.l}^{-1}$, multiply these concentrations by the factor 14/62

According to a recent study by the RIVM (Van Dijk-Looyaard, 1987), nitrate concentrations are rising at at least 62 water supplies and the sulphate content at 61 water supplies. Underground denitrification via pyrite plays an important role in the latter.

Problems with nitrate, either directly or indirectly, can probably be expected at 25 % of the groundwater supplies. It is expected that a nitrate concentration of $50 \text{ mg NO}_3^- \cdot \text{l}^{-1}$ will be reached at 9 pumping stations before the year 2000. In addition, this concentration has already been found at many boreholes on the site of the pumping stations. Figure 4.10. shows an example of the rising nitrate concentration for two pumping stations. Since 1984, these two companies have taken measures to reduce the nitrate concentration in the water supplied.

At many private wells the drinking-water standard is already far exceeded and concentrations of several hundred $\text{mg NO}_3^- \cdot \text{l}^{-1}$ are often found.

An increase in the nitrate concentration in the groundwater is also observed in nearly all the countries surrounding the Netherlands, particularly over the past 10 years. The main reason for this is an increase in intensive livestock farming and the use of chemical fertilizers.

Several million people in Europe are currently dependent on drinking water that exceeds the nitrate level of $10 \text{ mg NO}_3^- \cdot \text{N.l}^{-1}$ (WHO, 1985). This growing trend is likely to continue over the next few decades. The standard of $10 \text{ mg NO}_3^- \cdot \text{N.l}^{-1}$ will rarely be exceeded in drinking water prepared from surface water, although there may be problems locally (Warn, 1984).

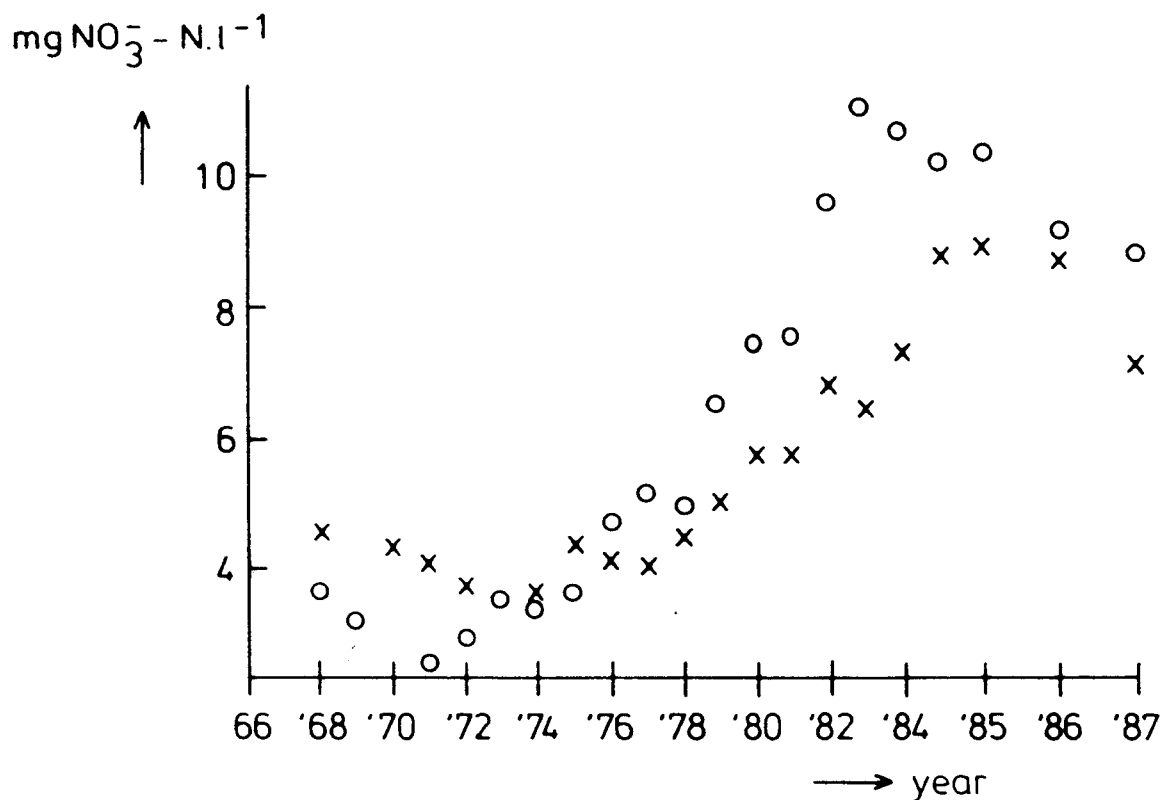


Figure 4.10 Nitrate concentration in the water supplied at the pumping stations Amby, Limburg (x) and van Heek, Gelderland (o) (RIVM data)

4.6. EXPOSURE LEVELS

On the basis of the concentrations given above, which have been summarized in table 4.16., exposure levels to man and (parts of) ecosystems can be estimated.

Exposure to human beings will depend primarily on the nitrate concentration in food, vegetables and drinking water.

The average volume of air inhaled daily is 12 m³, increasing to 40 m³ when moderately heavy work is performed (Rosenblatt et al., 1982). The daily intake of nitrate via respiration, calculated from the volume of breathing air required and the highest average 24-hour concentration (indoor air in house with gas cooker), will be 1 mg at most.

The daily average nitrate load via food intake amounts to about 143 mg (table 4.13.). The greater part of this is derived from vegetables. Large

daily variations can occur, depending on species, origin and method of preparation of the vegetable.

The daily consumption of drinking water is 2 litres (Van Wijnen, 1981; Zielhuis, 1981; Rosenblatt et al., 1982). Eighty per cent of the pumping stations supply drinking water with a nitrate concentration of $< 2.3 \text{ mg NO}_3^- \cdot \text{N.l}^{-1}$. The daily intake in these areas will be $4.5 \text{ mg NO}_3^- \cdot \text{N}$ at most. In 1982 the nitrate concentration in the drinking water supplied by the remaining pumping stations was $2.3\text{-}5.6 \text{ mg NO}_3^- \cdot \text{N.l}^{-1}$ (15 %) and $5.6\text{-}11.3 \text{ mg NO}_3^- \cdot \text{N.l}^{-1}$ (5 %) (CCRX, 1987).

The daily intake via drinking water in these areas is 11.3 mg and $22.6 \text{ mg NO}_3^- \cdot \text{N}$, respectively, at the most. As regards the aquatic ecosystems, the exposure level can be equated to the concentration in the surface water, and this is heavily dependent on various factors mentioned in section 4.3. For terrestrial ecosystems, too, the exposure level can differ widely.

4.7. SUMMARY AND CONCLUSIONS

In the soil, the nitrate concentration in soil moisture and groundwater is, apart from land usage (or more generally the immissions), determined to a large extent by the soil type and the hydrological situation. For example, hardly any nitrate is found under anaerobic conditions (peat areas) whereas in sandy areas tens of $\text{mg NO}_3^- \cdot \text{N.l}^{-1}$ are often observed, depending on the local situation. Forests have natural background concentrations of $0.2\text{-}0.5 \text{ mg NO}_3^- \cdot \text{N.l}^{-1}$ under aerobic conditions. These values can rise to tens of $\text{mg NO}_3^- \cdot \text{N.l}^{-1}$ in both agricultural areas and forests as a result of intensive livestock farming.

The large rivers contain naturally nitrate concentrations varying from 0 to $1 \text{ mg NO}_3^- \cdot \text{N.l}^{-1}$. At this moment the mean concentration in the Rhine is ca $4 \text{ mg NO}_3^- \cdot \text{N.l}^{-1}$. The concentrations are lower in summer than in winter. There are large differences between the small surface waters, which is partly related to soil type and land use. The concentrations in winter are about 10 times higher than in summer. The concentrations found in summer are generally between 0.1 and $0.5 \text{ mg NO}_3^- \cdot \text{N.l}^{-1}$. In winter, the concentrations range from 0.9 to 5 or more $\text{mg NO}_3^- \cdot \text{N.l}^{-1}$. Little is known about the occurrence of nitrate in the indoor air, although a relationship has been demonstrated with household appliances such as gas cookers. In the outdoor air, the average nitrate concentration in aerosol is $7 \text{ } \mu\text{g NO}_3^- \cdot \text{m}^{-3}$; an

average value of $4 \mu\text{g NO}_3^- \cdot \text{m}^{-3}$ was found for nitrate in the form of nitric acid. The concentrations in summer are about 1.5 times higher than in winter. The wet deposition of nitrate varies between 5.6 and 7.7 kg $\text{N} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$ and has more than doubled since 1960. The dry deposition of NO_x has been calculated to be 17 kg $\text{N} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$, on average, with a margin of uncertainty of 50 %; measurement data are not available.

As regards food, the highest nitrate concentrations are found in vegetables. Accordingly, around 120 mg of the average daily intake of about 143 mg of nitrate by man via food is derived from vegetables. For 80 % of the groundwater pumping stations, the daily intake by man via drinking water is on average 20 mg nitrate or less. This can rise to 100 mg nitrate for 5 % of the stations. The intake via the atmosphere (respiration) is less than 1 mg nitrate.

Table 4.16. Summary of levels of NO_3^- -N concentrations and contents in various parts of the environment in the Netherlands

<u>Soil</u>		<u>10 m -g.l.</u>	<u>shallow</u>
- groundwater	sandy areas	0.4 - 23 $\text{mg} \cdot \text{l}^{-1}$	2.2 - 62.6
	marine-clay areas	0.1 $\text{mg} \cdot \text{l}^{-1}$	
	river-clay areas	1.2 $\text{mg} \cdot \text{l}^{-1}$	2.9 - 21.7
	high moors	2.1 $\text{mg} \cdot \text{l}^{-1}$	
	low moors	0.1 $\text{mg} \cdot \text{l}^{-1}$	0.6 - 0.8
	loess areas	1.1 $\text{mg} \cdot \text{l}^{-1}$	
<u>Surface water</u>			
	sandy areas	0.2 - 5.0 $\text{mg} \cdot \text{l}^{-1}$	
	clay areas	0.1 - 3.2 $\text{mg} \cdot \text{l}^{-1}$	
	low moors	0.1 - 2.3 $\text{mg} \cdot \text{l}^{-1}$	
	sandy areas with intensive livestock farming	- 8.4 $\text{mg} \cdot \text{l}^{-1}$	
	North Sea	- ca 1 $\text{mg} \cdot \text{l}^{-1}$	
<u>Air</u>			
- indoor air(1) nitrate aerosol:			
	- dwellings with gas cooker	- 5.6 $\mu\text{g} \cdot \text{m}^{-3}$	
	- dwellings with electric cooker	- 1.8 $\mu\text{g} \cdot \text{m}^{-3}$	
	- offices	- 1.1 $\mu\text{g} \cdot \text{m}^{-3}$	
- outdoor air			
	nitrate aerosol	1.1 - 1.8 (max 27) $\mu\text{g} \cdot \text{m}^{-3}$	
	nitric acid	0.7 - 1.1 (max 9.9) $\mu\text{g} \cdot \text{m}^{-3}$	
	rainwater	400 - 550 $\text{mol} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$	
<u>Food</u>		ca 25 $\text{mg} \cdot \text{kg}_1^{-1}$	
<u>Drinking water</u>		- 11 $\text{mg} \cdot \text{l}^{-1}$	

(1) based on foreign data

5. EFFECTS

The toxic effects of nitrate, nitrite and nitrosamines will be discussed briefly in this chapter. For a more detailed review, including references, the reader is referred to the Nitrate Addendum in English.

5.1. HUMAN TOXICITY - NITRATE AND NITRITE

5.1.1. Chemobiokinetics and metabolism

Nitrate is absorbed by both humans and rats from the upper part of the digestive tract (Bartholomew and Hill, 1984; Witter et al., 1979a). Absorption is rapid: several researchers (Bartholomew and Hill, 1984; Ellen et al., 1982; Spiegelhalder et al., 1976; Turek et al., 1980) found peak values of nitrate in serum, saliva and urine of human subjects within 1-3 hours after ingestion of nitrate in food or drinking water.

Low levels of nitrate are normally found in body fluids and tissues (Witter and Balish, 1979; Fritsch et al., 1985).

In man and most experimental animals, but not in rats, nitrate is excreted selectively via the salivary glands into the saliva. Active carriers are probably involved in the transport of nitrate from blood to saliva, in which iodide and thiocyanate (SCN) act as competitive inhibitors (NAS, 1984); however, this active transport system is lacking in rats (as cited by Mirvisch (1983) from Cohen and Myant (1959)).

Nitrate can also be present in milk but, unlike salivary excretion, the transport of nitrate into milk is not an active process (Green et al., 1982).

Nitrate is partly converted to nitrite by microorganisms present in saliva. Of the ingested nitrate, 4-7 % is recovered as nitrite in the saliva (Eisenbrandt et al., 1980; Stephany and Schuller, 1980).

No bacterial nitrate reduction occurs at low pH values due to poor bacterial growth; it is assumed that this therefore does not take place in the stomach of most monogastric animals, including man (Wright and Davison, 1964; Mirvish et al., 1975). High gastric pH values and occasionally corresponding high nitrite values have, however, been observed in patients with gastric cancer and stomach ulcers, in patients with atrophic gastritis and in patients treated with cimetidine and antacids (Correa et al., 1975; Ruddell et al., 1976; Schlag et al., 1982; Bartsch et al., 1984). Children

under the age of 3 months are also very susceptible to gastric bacterial nitrate reduction because they produce very little gastric acid (as cited in a review by Ellen and Schuller, 1983). Gastrointestinal infections, which are common in children, can cause a further increase in nitrate reduction to nitrite.

Nitrate reduction in saliva is negligible in rats (Witter et al., 1979a; Til, 1986), which is probably due to the low secretion of nitrate by the salivary glands of this species. The half-life of nitrate in the human body following ingestion is about 5 hours (Wagner et al., 1983b).

Of single oral doses of 25-170 mg potassium nitrate administered to human subjects, 65-70 % was excreted in the urine, irrespective of the dose. Excretion was maximal 5 hours after ingestion and returned to baseline levels within 18 hours (Bartholomew and Hill, 1984). Larger single oral doses (7-10.5 g) of ammonium nitrate resulted in an average urinary nitrate excretion of 75 % within 24 hours, while small quantities of nitrite were also found in 26 % of the samples (Ellen et al., 1982). One study reported that 80-100 % of the average nitrate intake by children was excreted in the urine, but the amount ingested was not specified (Turek et al., 1980). In another study with young healthy children it was found that with low nitrate intake (about 2-7 mg NO_3^- plus NO_2^- per day), urinary excretion (3-16 mg, mean 8.7 mg NO_3^- per day) was the same or higher than the amount ingested (Hegesh and Shiloah, 1982). However, this excretion probably included endogenously synthesized nitrate.

Several authors reported a sharply increased nitrate excretion in humans (0.3-1.9 mmol excreted in urine per day) when nitrate intake was low (less than 0.25 mmol per day) (Bartholomew and Hill, 1984; Green et al., 1981; Lee et al., 1986; Tannenbaum and Green, 1981; Wagner et al., 1983b). When large amounts of ^{15}N -labelled nitrate were ingested (up to 3.5 mmol), the urinary excretion of unlabelled nitrate was still considerable (0.7-1.3 mmol against 0.2 mmol intake, Green et al., 1981). Ellen and Schuller (1983) calculated that up to 20 % of this excessive excretion could have originated from the inhalation of NO_2^- and NO_3^- from indoor and outdoor air and cigarette smoke. The remaining excess urinary nitrate, up to 1 mmol per day, is very probably synthesized endogenously. Conclusive proof of nitrate biosynthesis was furnished by Dull and Hotchkiss (1984) for ferrets and by Saul and Archer (1984) and Wagner et al. (1983a) for rats. Ingestion of ^{15}N -labelled ammonium salts was invariably followed by excretion in the urine of small amounts of ^{15}N -labelled nitrate. Although bacterial activity

is not essential for this synthesis in animals, it can certainly enhance nitrate biosynthesis; the occurrence of gastrointestinal infections may be important here.

Wagner et al. (1983), for example, found a ninefold increase in urinary excretion of nitrate in rats after intraperitoneal administration of a bacterial endotoxin; Stuer and Marletta (1985) showed that infection with Mycobacterium bovis increased the excretion of nitrate in mice more than fortyfold. Hegesh and Shiloah (1982) found a sharply increased urinary nitrate excretion in infants with acute diarrhoea, from a normal average of 8.7 to an average of 39 mg NO_3^- per 24 hours, at a constant low intake of 2 to 7 mg NO_3^- plus NO_2^- daily.

As a result of reaction with the gastric contents (e.g. nitrosation, WHO, 1985; see also 5.1.4. Nitrosation) or reduction by the gastrointestinal flora, a large proportion of the nitrate entering the gastrointestinal tract can have disappeared before absorption could take place.

Gastric absorption of nitrite has been observed in rats and mice in radioactivity studies and studies in which the gastric contents were analyzed (Witter et al., 1979b; Mirvish et al., 1975). No data are available concerning the absorption of nitrite in man. In view of the observations that part of the ingested nitrate is converted to nitrite in the oral cavity and stomach, and since high methaemoglobin levels were found in children who had ingested large quantities of nitrate (Shuval and Gruener, 1972), nitrite is apparently absorbed from the gastrointestinal tract.

Nitrite can be reduced in the gastrointestinal tract with the formation of nitric oxide (NO) (Newmark and Mergens, 1981).

Absorbed nitrite is rapidly oxidized in the blood to nitrate (Witter and Balish, 1979; Fritsch et al., 1985; Parks et al., 1981). The organism itself probably contributes to this oxidation; it is not the result of bacterial action (Witter and Balish, 1979). Oxidation of nitrite to nitrate may also occur in the stomach before absorption occurs, as was demonstrated in vitro for mice. Under in vivo conditions, however, nitrite is probably absorbed from the stomach before large amounts of nitrate have been formed (Friedman et al., 1972).

Nitrite in the blood stream (both exogenous nitrite and nitrite formed from exogenous and/or endogenously synthesized nitrate) brings about oxidation of haemoglobin to methaemoglobin. In this reaction, the Fe_2^+ atom in the haem group is oxidized to Fe_3^+ and nitrite binds firmly to this oxidized

haem, so that the transport of oxygen is inhibited (Jaffe,1981). Methaemoglobin formation is reversible at low nitrite levels.

Infants are very susceptible to nitrite-induced methaemoglobin formation (Ellen and Schuller,1983), since:

- foetal haemoglobin, which comprises initially 60-80 % of total haemoglobin and decreases to 20-30% within three months, is converted to methaemoglobin more rapidly than the adult form;
- NADHP methaemoglobin reductase, which catalyzes the reduction of methaemoglobin back to haemoglobin, is normally deficient in neonatal erythrocytes.

5.1.2. Animal studies, nitrate

Toxicity

The acute oral LD50 values for mice ranged from 2480 to 6250 mg sodium nitrate per kg body wt, and for rats from 4860 to 9000 mg sodium nitrate per kg body wt; for rabbits the LD50 was 1600 and for cows 450 mg sodium nitrate per kg body wt. It can be concluded from these data that cows are the most sensitive to nitrate. It is known from kinetic and metabolic studies that the toxicity of nitrate is largely determined by its conversion to nitrite and the subsequent formation of methaemoglobin. This is in agreement with the lower LD50 values found in ruminants, where the conversion of nitrate to nitrite is considerably greater than in non-ruminating calves and rodents. These observations are corroborated by the finding that administration of the total dose to cows in smaller doses over a 24-hour period reduced the toxicity considerably (450 versus >970 mg sodium nitrate per kg body wt). Also, a rather high subacute toxicity (200 mg per kg body wt still had toxic effects) was found by Nighat et al. (1981) in rabbits, the nitrate being administered as a pulse dose in gelatin capsules.

The long-term study by Lehman (1958) in rats is considered the most appropriate for establishing a no-toxic effect level; the results were confirmed by Til et al. (1985 a and b). This study estimated the no-effect level for rats to be 500 mg sodium nitrate per kg body wt.

Carcinogenicity

Part of the carcinogenicity studies are regarded as unsatisfactory on account of duration of the experiment, number of animals used, route of

administration, lack of information about the presence of nitroso compounds in the diet, or precisely because of the presence of nitroso compounds in the diet. Nevertheless, it can be concluded from both the only appropriate study by Maekawa et al. (1982) and the studies which were conducted not entirely in accordance with proper scientific method, that nitrate did not exhibit any carcinogenic activity in experimental animals.

Mutagenicity

Nitrate is not mutagenic in bacteria and in mammalian cells in vitro. Chromosomal aberrations were induced in in vivo rat bone marrow experiments, but it cannot be ruled out that the formation of nitroso compounds was responsible for this chromosomal damage. It can be concluded that nitrate is probably not mutagenic.

Reproduction toxicity/teratogenicity

A no-toxic effect level of 1000 mg potassium nitrate per litre of drinking water, equivalent to 1000 mg per kg body wt, can be deduced from reproduction studies in guinea pigs.

No adequate data exist on a possible teratogenic effect of nitrate.

5.1.3. Animal studies, nitrite

As stated earlier on the basis of toxicity and kinetic studies, the toxicity of nitrate depends largely on its conversion to nitrite. Conversion in the rat differs from that in man, it being 4-7 % in the latter. Therefore, the no-toxic effect level of nitrate can probably best be calculated from the no-effect level of nitrite in the rat and the percentage of conversion known for man.

Toxicity

The acute oral LD50 value for mice ranged from 175 to 220 mg sodium nitrite per kg body wt while the LD50 for rats was 85 mg per kg body wt. The toxicity of nitrite decreased when a single dose of nitrite was administered as a number of smaller doses. It can be estimated from the chronic toxicity study in rats (Shuval and Gruener, 1972) that the no-toxic effect level is 100 mg sodium nitrite per l, or 10 mg sodium nitrite per kg body wt. The next higher dose level tested in this study was however 10 times higher.

Carcinogenicity

Many studies on mice and rats concerning the possible carcinogenicity of nitrite, with administration of nitrite in feed or drinking water, were likewise inadequate, either on account of duration of the experiment, number of animals used, absence of information about the content of nitroso compounds in the diet, or because of the fact that the only group receiving a nitrite dose was intended to be a control group in studies designed to investigate the carcinogenic effects of simultaneous administration of nitrite and a nitrosatable amine.

The original conclusion from a large-scale carcinogenicity study in rats carried out by the FDA (Newberne, 1978; 1979), in which nitrite was administered via feed or drinking water, was that there was an increased incidence of lymphomas. The study was re-analyzed by an Interagency Working Group of the U.S. government, which concluded that there was no increase in tumour incidence, either of lymphomas or of other types of tumours (FDA, 1980a and b).

Although a small number of studies with nitrite showed a slight increase in tumour incidence, probably as a result of the nitrosamine present in the diet fed to the mice and rats, the final conclusion can nevertheless be that nitrite itself is not carcinogenic.

Mutagenicity

Nitrite is mutagenic and causes morphological changes in in vitro systems. Mutagenic activity was also found in a combined in vivo-in vitro experiment with Syrian hamsters. In vivo experiments yielded conflicting results.

The mutagenesis observed in some in vitro systems is probably due to deamination, while in the in vivo experiments endogenous formation of nitroso compounds may play a role.

Reproduction toxicity/teratogenicity

Nitrite exhibits foetotoxic effects. Foetotoxicity experiments on rats in which nitrite was administered via drinking water showed effects at both levels tested (200 and 300 mg per kg body wt), while no effects were observed in foetotoxicity and teratogenicity studies in rats which received a dose of 200 mg sodium nitrite per kg body wt via the feed.

5.1.4. Nitrosation

Nitrosation by reaction of nitrite with a nitrosatable compound is a pH-dependent process. Nitrosation can occur, for example, during storage and processing of food, cosmetics and tobacco products.

Endogenous nitrosation has been investigated both in vitro and in vivo. Nitrosation of various amino and amido compounds and of several food constituents (especially fish) was demonstrated in gastric simulation studies.

In vivo studies in laboratory animals clearly established the occurrence of nitrosation.

Several qualitative studies showed that endogenous nitrosation also occurs in man. For example, traces of volatile nitrosamines (dimethylnitrosamine and diethylnitrosamine) were detected in the gastric contents of humans after simultaneous consumption of nitrate-rich vegetables and fish (fish contains amines in the form of nitrosatable compounds).

Ohshima and Bartsch (1981) performed a quantitative determination of endogenous nitrosation in man. The urinary excretion of nitrosoproline following oral intake of nitrate and proline was measured. The total amount of nitrosoproline excreted into the urine increased linearly with the proline dose and exponentially with the nitrate dose. On average 23.3 ug nitrosoproline was formed at the highest dose of nitrate (325 mg) and proline (500 mg). When nitrate intake was 195 mg or 130 mg, the amount of nitrosoproline formed was 3 ug and 2 ug respectively. Following consumption of 65 mg nitrate together with 500 mg proline, the amount of nitrosoproline measured was negligible. It should be noted here that dimethylamine and diethylamine are nitrosated less rapidly than proline, while morpholine and pyrrolidine are nitrosated more rapidly than proline.

5.1.5. Observations in man

Toxicity

The lowest oral lethal dose of nitrate for adults reported in the literature is 80 mg NO_3^- per kg body wt. A more realistic dose of 20 g, or 330 mg NO_3^- per kg body wt, can be estimated from more recent data. However, the toxicity of nitrate in humans depends on the conversion of nitrate to nitrite.

The lowest oral acute lethal dose of nitrite reported for man varied between 33 and 250 mg per kg body wt, the lowest values applying to very young children and the elderly. Nitrite is also more toxic to very young children than to adults because of the relatively higher methaemoglobin concentrations produced in their blood. The lowest toxic dose mentioned was 1 mg NO_2^- per kg body wt, while in another study 0.5-5 mg NO_2^- per kg body wt did not cause any toxic effects.

Patients with achlorhydria and/or stomach lesions and infants up to the age of 3 months can be distinguished as risk groups for the toxicity of nitrate. Assuming that in these very young children 80 % of the nitrate is reduced to nitrite, and using the formation of 10 % methaemoglobin or more as the criterion, then the lowest oral toxic dose of nitrate for this risk group can be calculated from that of nitrite as being 1.5-2.7 mg NO_3^- per kg body wt. In reported cases of methaemoglobinaemia in infants the intake of nitrate was higher (37.1-108.6 mg $\text{NO}_3^- \cdot \text{kg}^{-1}$ body wt). Of all reported cases of methaemoglobinaemia in infants, 97.7 % occurred in areas where the drinking water contained more than 10-20 mg $\text{NO}_3^- \cdot \text{N}$ per l (equivalent to >44.3-88.6 mg NO_3^- per l; WHO, 1984; 1985). The evaluation of cases of methaemoglobinaemia in relation to nitrate intake is hampered by the often simultaneous occurrence of bacterial contamination of the drinking water, and bacterial infections which can influence both the reduction of nitrate to nitrite and the endogenous synthesis of nitrate. Hegesh and Shiloah (1982) found a significant increase in nitrate content and, correlatively, methaemoglobin content in the blood of infants with acute diarrhoea, although the intake of nitrate and nitrite was low in all cases (2-7 mg per day; see also 5.1.1.).

In one experimental study different nitrate doses were given to infants. Oral treatment of healthy children, aged from 11 days to 11 months, with 50 or 100 mg NO_3^- per kg body wt for 18 days produced methaemoglobin concentrations of 5.3-7.5 % without accompanying cyanosis. Administration of 100 mg NO_3^- per kg body wt to 6 to 7-week-old children recovering from previous methaemoglobinaemia resulted in 11 % methaemoglobin in combination with cyanosis (Cornblatt and Hartmann, 1984). This study gave no control data; the normal blood methaemoglobin value is however about 1-2 % (WHO, 1985).

No data are available on the toxicity of nitrate to adults following short-term or long-term exposure except for epidemiological studies on the relationship between nitrate or nitrite intake and tumour incidence.

Carcinogenicity

Several countries have reported a relationship between high levels of nitrate intake and stomach cancer, and a connection was found between exposure to nitrate and cancer of the stomach in areas of Chile, Columbia, Denmark, England, Hungary, Italy and Japan (Hartmann, 1983). The proofs, however, are merely suggestive and a few are even contradicted by other studies (Davies, 1980; Armijo, 1981a and b). Several other studies found a negative association.

The majority of epidemiological studies usually calculate nitrate intake from questionnaires, or use nitrate levels in saliva, gastric juice or urine as a measure of the nitrate intake. However, several studies found no correlation between the risk of gastric cancer and the salivary nitrate concentration, although there was a correlation with the urinary nitrate (Cuello et al., 1976; Eisenbrand et al., 1980).

Many complicating factors should be taken into account in epidemiological and related studies. However, full information on these factors is often not available. Information about the presence of carcinogenic nitroso compounds in the diet or other lifestyle factors is not included in any of these studies. The relevance of the current nitrate levels - exogenous or in saliva - to the current incidence of stomach cancer is also disputable, because of the long induction period of tumours.

Factors other than nitrate which are responsible for the degenerative changes in the protective gastric mucosa before carcinogens cause damage are possibly more important. Differences between countries in, for example, the consumption of salted or fermented foodstuffs along with high gastric cancer mortality, such as Japan and China, may play a decisive role in this process and may explain the inconsistent findings.

In conclusion, no definitive relationship has been found between the estimated nitrate intake and the development of stomach cancer in normal healthy persons, although certain studies pointed to such a link. No increased gastric cancer incidences were observed in occupational situations after exposure to nitrate or nitrite.

People with stomach lesions or disorders, particularly those with atrophic gastritis, patients with pernicious anaemia, and persons treated with cimetidine or antacids are special risk groups in which a correlation between nitrate or nitrite consumption and the incidence of stomach cancer cannot be ruled out.

For both healthy persons and certain risk groups, the possible correlation between nitrate and nitrite intake and stomach cancer is based on the possible endogenous formation of carcinogenic N-nitroso compounds. Combining this with the facts that for this formation to occur also an amine or amide must be present in relevant concentrations - as discussed earlier - and that neither nitrate nor nitrite proved to be carcinogenic in animal studies under well-defined conditions, the conclusion can be that nitrate and nitrite themselves do not have carcinogenic properties.

5.2. HUMAN TOXICITY - NITROSAMINES

People can be exposed to nitrosamines via ingestion, inhalation and dermal contact. In general, only traces of nitrosamines are found in the environment. In addition, nitrosamines can be formed endogenously from various nitrosatable compounds.

5.2.1. Chemobiokinetics and metabolism

Nitrosamines require metabolic activation to reactive metabolites to exert their biological effects. It is generally accepted that the primary reaction is alpha-hydroxylation with subsequent dealkylation of the reactive intermediate to an alkyl diazohydroxide and, finally, formation of an alkylating agent resulting in alkylation of cellular macromolecules. In addition to activation, deactivation also occurs (see figure II in Appendix).

5.2.2. Carcinogenicity

Most nitrosamines are carcinogenic in all animal species examined and induce tumours in organs and cell types which differ from one species to the next (=organotropism). This organotropism of the carcinogenic effect may concern a number of organs or organ systems, although the liver is the principal target organ. The organotropism depends not only on the chemical structure of the molecule but also, for example, on the size of the dose administered and the strain used within the same animal species. The route of administration plays a minor role here.

It is highly likely that most nitrosamines are also carcinogenic in man. Of the 14 nitrosamines evaluated, nitrosothiazolidine, nitrosoanabutine and

4-(N-nitrosomethylamino)-4-(3-pyridyl)butanal were tested insufficiently to enable evaluation of their carcinogenic activity.

5.2.3. Mutagenicity

Nitrosamines exhibited weak mutagenic activity in bacterial tests in the presence of a metabolic activation system. Distinct reactions were observed under special conditions, such as: a) preincubation in a liquid; b) metabolic activation by hamster liver fractions in place of rat liver fractions; c) metabolic activation by primary hepatocytes instead of S-9 liver mix, and d) weakly acidic instead of neutral medium.

Human liver fractions activated the cyclic nitrosamines nitrosopiperidine, nitrosomorpholine and nitrosopyrrolidine in bacterial systems but not the dialkylnitrosamines dimethylnitrosamine, dipropylnitrosamine and dibutylnitrosamine.

For the dialkylnitrosamines an inverse relationship was found between mutagenic potency in the Salmonella test and carcinogenic potency in animal studies. A direct relationship was found for the cyclic nitrosamines.

In all other in vitro systems (fungi, mammalian cells), mutagenic activity was observed upon metabolic activation. Species differences in bioactivation were also found in these systems. As regards the induction of genetic mutations, the activity of the dialkylnitrosamines decreased with increasing chain length. The chromosomal aberrations induced by dimethylnitrosamine and diethylnitrosamine were mostly chromatid exchanges whereas dipropylnitrosamine and dibutylnitrosamine induced mainly breaks. Nitrosamines showed distinct activity in the sex-linked recessive lethal test in Drosophila.

In vivo studies with nitrosamines demonstrated mutagenic activity in somatic cells at relatively high doses (compared to LD50 values). No effects were observed in germ cells.

Nitrosamines caused DNA damage in somatic cells both in vitro and in vivo. Nitrosamines induced single-strand breaks in DNA of somatic cells and nitrosomorpholine induced furthermore also double-strand breaks.

In vivo studies with nitrosamines showed inhibition of DNA synthesis, but no DNA damage, in germ cells.

Nitrosothiazolidine and nitrosamines present in tobacco were tested insufficiently to evaluate their possible mutagenic activity.

5.2.4. Observations in man

Acute toxicity of dimethylnitrosamine has been reported in several older publications (liver cirrhosis and necrosis) in occupationally exposed persons. These findings were confirmed in more recent publications concerning several cases of poisoning with dimethylnitrosamine.

However, it is difficult, if not impossible, to demonstrate a cause/effect relationship between low-level exposure to nitrosamines and the incidence of certain cancers in the general population. This is because of the insensitivity of the epidemiological parameters known to date and the lack of an unexposed population group which could be used as a control. Furthermore, individual exposure to endogenously synthesized nitrosamines is influenced by particular dietary components. Data from a number of epidemiological studies are merely suggestive.

More conclusive are the studies on snuff (smokeless tobacco) dippers (snuff dipping is the oral use of snuff whereby the tobacco is not chewed but is retained at the same place in the mouth) and smokeless-tobacco chewers. There is sufficient evidence that the oral use of snuff of the types commonly used in some southern states of North America and in Scandinavia is carcinogenic to humans. There is only "limited evidence" for a link between chewing smokeless tobacco in these areas and tumour induction.

There is inadequate proof of a relation between the inhalation of smokeless tobacco through the nose (snuffing) and the induction of tumours.

5.3. ECOSYSTEMS

5.3.1. Aquatic environment

The data given in this section on the toxicity of nitrate, nitrite and ammonia in the aquatic environment are based on the data included in chapter 3 of the Appendix. A full overview of the references is included in this appendix.

Aquatic toxicity, nitrate

The toxicity of nitrate is highly dependent on the cationic composition of the water. According to Canton and Slooff's proposal (1979), nitrate can be classified as having very low toxicity, with L(E)C50 values of $>100 \text{ mg NO}_3^- \cdot \text{l}^{-1}$ (short-term) and NOL(E)C values of $>1 \text{ mg NO}_3^- \cdot \text{l}^{-1}$ (long-term), with

sodium nitrate in the water. This holds for both freshwater and marine organisms.

With potassium nitrate and nitric acid as test substance, nitrate is slightly toxic ($10 < \text{LC50} < 100 \text{ mg NO}_3^- \cdot \text{l}^{-1}$) to the freshwater organism Daphnia magna and the marine organism Ophryotrocha diadema, with short-term exposure. The other available data also indicate that potassium nitrate has very low toxicity.

Aquatic toxicity, nitrite

The toxicity of nitrite depends heavily on a number of environmental factors. A relatively high pH and a high chloride content (especially at high Ca_2^+ concentrations) both have a protective effect.

According to Canton and Slooff's proposal (1979), nitrite is highly toxic to freshwater organisms in water with a low chloride concentration at short-term exposure ($\text{LC50} < 1 \text{ mg NO}_2^- \cdot \text{l}^{-1}$). On the basis of data on long-term exposure, nitrite appears to be slightly toxic to freshwater fish, with an NOEC of $0.1\text{-}1 \text{ mg NO}_2^- \cdot \text{l}^{-1}$.

Data on short-term exposure to nitrite suggest that it is moderately toxic to marine organisms ($1 < \text{LC50} < 10 \text{ mg NO}_2^- \cdot \text{l}^{-1}$); at long-term exposure, nitrite is very slightly toxic to marine algae ($\text{NOEC} > 1 \text{ mg NO}_2^- \cdot \text{l}^{-1}$).

Aquatic toxicity, ammonia

The toxicity of total ammonia ($\text{NH}_3 + \text{NH}_4^+$) is greatly dependent on the amount of "unionized ammonia" (NH_3) in the water. This amount and the consequent toxicity of ammonia in the environment is enhanced by an increase in pH and temperature.

According to Canton and Slooff's proposal (1979), NH_3 can be classified as being highly toxic to freshwater organisms at short-term exposure ($\text{LC50} < 1 \text{ mg NH}_3 \cdot \text{l}^{-1}$). NH_3 is also very toxic to freshwater organisms at long-term exposure ($\text{NOEC} < 0.01 \text{ mg NH}_3 \cdot \text{l}^{-1}$). NH_3 is very toxic to marine organisms at short-term exposure and moderately toxic ($0.01 < \text{NOEC} < 0.1 \text{ mg NH}_3 \cdot \text{l}^{-1}$) at long-term exposure.

Eutrophication of surface water

As a logical outcome of the processes underlying the nitrogen cycle, the utility of setting limits for nitrate only can be seriously questioned. Under normal conditions all biologically "active" forms of nitrogen (organic N, ammonia, nitrate and nitrite) are continuously being formed

from each other. It therefore seems more sensible to formulate target values for the total concentration of biologically active nitrogen components. Target values for nitrate can be derived from these, assuming worst-case conditions. In the following evaluation, the target maximum nitrogen concentrations in the surface water have been deduced from observations made during the period before there was excessive eutrophication. It is apparent from data produced by Lindeboom (1986) that it is only since 1945 that the nitrate concentration in the Rhine has been increasing sharply. The oldest known observations (for the rivers from 1874 to 1942 and for fens, dunal lakes and streams around 1960) show a total nitrogen concentration of about 1 mg.l^{-1} both in the large rivers and lakes, canals and ditches fed by them, and in the water bodies fed by precipitation or groundwater. The naturally more eutrophic river water has a higher nitrate level ($0.4 \text{ mg N.l}^{-1} = 1.8 \text{ mg NO}_3^-. \text{l}^{-1}$) than the more oligotrophic fens, streams and dunal lakes ($< 0.01 \text{ mg N.l}^{-1} = < 0.04 \text{ mg NO}_3^-. \text{l}^{-1}$). For the Dutch coastal waters, a natural nitrate concentration of $0.4 \text{ mg NO}_3^-. \text{l}^{-1}$ can be derived from historical data.

5.3.2. Terrestrial environment

The possible effects of increased nitrate concentrations or nitrate loading on terrestrial ecosystems have been described on the basis of literature data. In the first instance, this does not concern toxic effects on plant and animal but rather structural changes in the functioning of the material cycles in these ecosystems. As nitrate is one of the principal nutrients, the emphasis will be especially on the effect of eutrophication on soil and groundwater resulting from the total nitrogen burden. To that end, the nitrogen fluxes and quantities in the ecosystem compartments have been quantified. Owing to the rapid interconversion of N components, there are virtually no mass balances for NO_3^- -N fluxes and quantities in terrestrial ecosystems.

The analysis performed compared the total nitrogen deposition and that of the individual components with the nitrogen fluxes occurring in the undisturbed system. The effect-studies on which this analysis is based can be divided into two categories, of which the first comprises fertilization studies in which often a relatively high, single nitrogen dose is applied. The second consists of field studies which signal deviations in the functioning of ecosystems or components thereof resulting from increased

(continuous) nitrogen deposition. This often makes interpretation of effects a complex enterprise, partly because of the occurrence of variation in other factors affecting the sensitivity of ecosystems spatially and temporarily.

Four types of terrestrial ecosystems (forest, heath, grassland, dune) have been analyzed. Figure 5.1. shows the observed qualitative effects for the first three types of ecosystem.

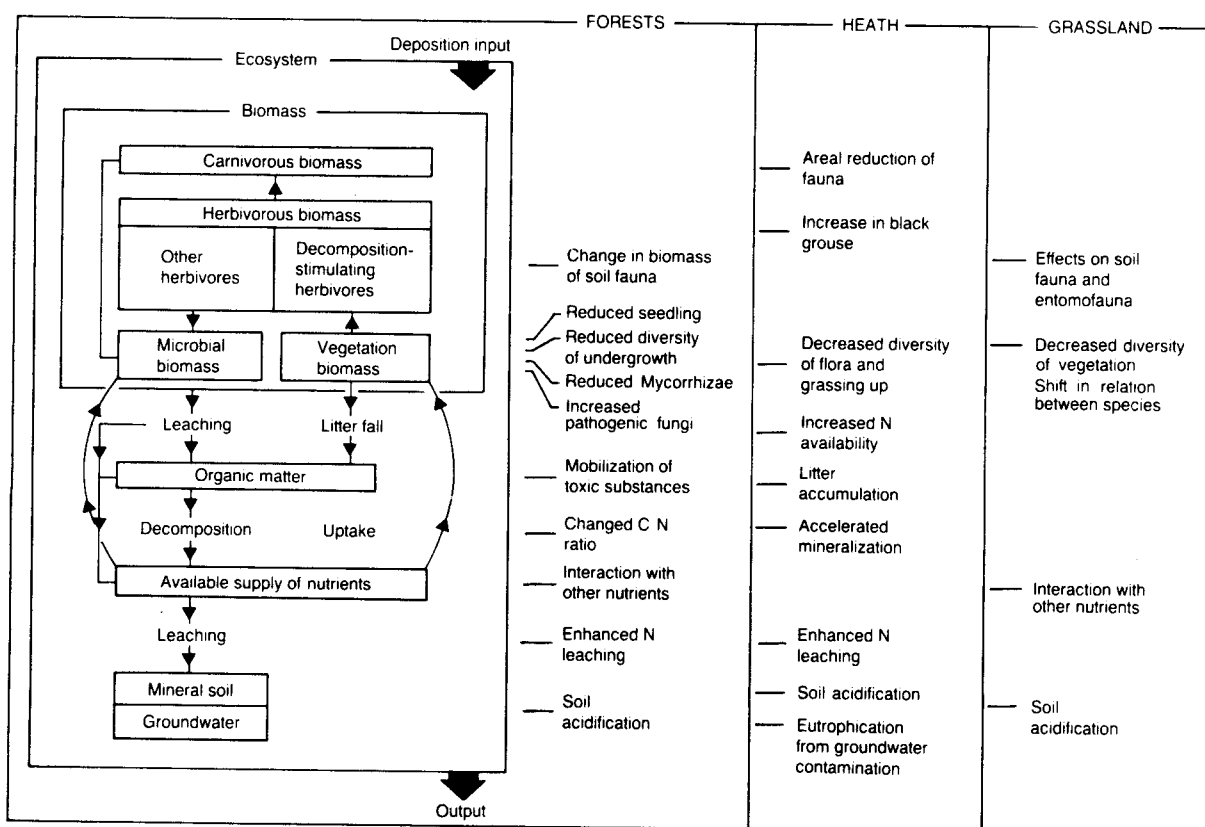


Figure 5.1. Summary chart of the effects of N loading, and their sites of action, on ecosystem compartments and processes in forests, heath and grassland.

Forest ecosystems are classified by dominant tree type and soil type. Important parameters in connection with extra nutrient input originating outside the system are uptake, litter fall, leaching and annual fixation by the vegetation. Age and productivity of the system play an important role in the degree of flexibility as regards the load to be processed. Uptake ranges from 45 to 123 kg N.ha⁻¹.yr⁻¹ for deciduous forests. The amount of N entering the decomposition system annually via cycling (litter) varies between 29 and 79 kg.ha⁻¹. It is striking how very low is the extent of leaching (1-4 kg.ha⁻¹.yr⁻¹), as observed in an undisturbed deciduous forest. Uptake by coniferous forests ranges from 34 to 67 kg.ha⁻¹.yr⁻¹, while 14-58 kg N.ha⁻¹ enters the decomposition subsystem with the annual litter fall. Here too there is very little leaching (0.5-1.0 kg N.ha⁻¹.yr⁻¹).

As a result of the filtering activity of forests, the vegetation front of a forest stand in particular will receive a higher N input than the interior of a forest. The effects described (figure 5.1.) will consequently first manifest themselves at the edge of a forest. For the same reason, the effects described will develop at an earlier stage in a forest with an open structure than in a dense forest.

Based on the uptake and annual fixation of nitrogen in forests, the annual deposition should amount to no more than half the current deposition, viz. 20-30 kg N.ha⁻¹.yr⁻¹. The permissible load for coniferous forests, assuming an integral ecosystem approach, is lower than that for deciduous forests. Here, it is assumed (owing to the lack of data) that N sources other than atmospheric deposition are nonexistent, which need not be so in reality.

Since the extent of leaching observed naturally in undisturbed systems is small, increased NO₃⁻-N leaching into the groundwater may be expected especially in forests on sandy soil at loads in excess of around 50 kg N.ha⁻¹.yr⁻¹; however, this is based on measurements in soils intended for agricultural purposes. A survey conducted in 1985 of nitrate contents in the shallow groundwater below forest stands in the province of Utrecht found that in about 75 % of the cases the nitrate content was higher than expected on the basis of the flux study.

In addition, changes in the composition and diversity of the soil fauna, which has an influence on the functioning of the ecosystem, are observed caused by a variety of N compounds. The observations concerning soil fauna are based on fertilization studies in which a relatively high, single dose

of artificial or animal manure is applied, and do not permit quantitative statements.

As regards the heath ecosystem, a distinction should be made between dry heath with Calluna vulgaris (ling) as the dominant species and moist heath with Erica tetralix (bell-heather) as the dominant flower element. Important parameters in connection with extra nutrient input originating outside the system are uptake, total litter fall, leaching and annual fixation by the vegetation. The heath's age is also important in connection with the magnitude of these fluxes. In the Dutch situation, the NO_3^- -N content of soil with an Erica vegetation was found to be very low. The levels fluctuated around the limit of detection and were invariably $< 0.5 \text{ kg N.ha}^{-1}$. Uptake by the relevant Erica vegetation ranged from 34 to $107 \text{ kg N.ha}^{-1}.\text{yr}^{-1}$, while uptake by Calluna vegetations (foreign research) varied between 13 and $25 \text{ kg N.ha}^{-1}.\text{yr}^{-1}$. Leaching into the groundwater below these Calluna vegetations amounted to $2\text{--}18 \text{ kg NO}_3^- \text{--N.ha}^{-1}.\text{yr}^{-1}$; this would be around $2 \text{ kg NO}_3^- \text{--N.ha}^{-1}.\text{yr}^{-1}$ for an Erica vegetation in the Netherlands.

The most striking effect of an increased nitrogen burden on heath is grassing up, which is already occurring on a large scale. This can be ascribed to the shift in the competitive relation between fast-growing grasses and slow-growing heather as a result of the increased N availability. Nitrogen is the primary growth-limiting nutrient in heath ecosystems. An increased N burden leads, through an increased nutrient content of the vegetation, to a higher plague frequency of black grouse. The resulting open spaces in the heath vegetation then grass up. Grassing starts to occur at N loads from around $14 \text{ kg N.ha}^{-1}.\text{yr}^{-1}$ on dry heath and from around $20 \text{ kg N.ha}^{-1}.\text{yr}^{-1}$ on moist heath. Effective nitrogen management is imperative here. On the basis of uptake and annual fixation, maximum N loads should be in the same order of magnitude as the above values.

In moist tracts of heath in particular, the quality of the groundwater is of overriding importance because of the need for a permanently high water table. Leaching of nitrate from adjacent agricultural land into groundwater that can reach the moist heath will lead to eutrophication.

Eutrophic groundwater may also be strongly influential in moist lean grasslands. In lower-lying grasslands, e.g. in the valleys of brooks, surface runoff from adjacent cultivated land can play a part. In relatively undisturbed grassland systems, an increased N burden will lead especially

to less diversity and species abundance of the vegetation. There is a reduction or shift in the assemblage of species of soil fauna and entomofauna at $> 50 \text{ kg N.ha}^{-1}.\text{yr}^{-1}$, possibly affecting the functioning of the decomposition process and of material cycles.

The nutrient supply in the dune ecosystem is generally poor in undisturbed systems with the exception of locations where humus or litter has accumulated. Phosphorus is the limiting nutrient in dune ecosystems except for dunal grasslands where nitrogen may also be a limiting factor. The buffering ability of chalk-rich dunes against acidification is reasonably good, but it is naturally zero in dunes low in chalk. It will therefore take longer before increased N deposition has effects on the vegetation of dune ecosystems, with the exception of the above-named dunal grasslands and low-chalk dunes. There are few reports describing effects caused specifically by nitrate or total N on ecosystem elements in Dutch dune areas.

5.4. SUMMARY AND RISK ASSESSMENT

5.4.1. Human toxicity

Experimental studies showed that ingested nitrate can give rise to an elevated methaemoglobin blood level in both healthy young children after short-term intake and adult animals following long- and short-term intake. Data on clinical cases of methaemoglobinaemia in infants cannot be evaluated in relation to nitrate ingestion because more factors often play a role here, such as bacterial contamination of drinking water and gastrointestinal infections in infants.

Apart from epidemiological data on tumour incidence, no data are available on the effects of semichronic or chronic ingestion of nitrate by man. For this reason a no-toxic effect level of $500 \text{ mg sodium nitrate.kg}^{-1} \text{ body wt}$ could be derived from a chronic feeding study with rats, in which a slight growth inhibition was observed at the next higher dose of $2500 \text{ mg sodium nitrate.kg}^{-1} \text{ body wt}$. However, the biokinetics and metabolism of rats and humans differ so that the rat has to be regarded as a less suitable animal species for studying the toxicity of nitrate. Effects were observed in a subacute study in rabbits, but this study was considered less relevant to a risk assessment because nitrate was administered as a pulse dose by means

of gelatin capsules, whereas humans ingest daily relatively small amounts of nitrate in food and drinking water. The toxicity of nitrate is determined chiefly by the amount of nitrite formed from nitrate, so that it would seem more appropriate to calculate the no-toxic effect level from the conversion of nitrate to nitrite in man and the no-toxic effect level in rats (see below).

Nitrate is nonmutagenic in bacteria and mammalian cells in vitro. In in vivo experiments chromosomal aberrations were induced in rat bone marrow, but it cannot be ruled out that the nitroso compounds formed were responsible for the chromosomal damage observed. It can be concluded from animal studies that nitrate itself does not have carcinogenic properties. Children up to 3 months of age should be recognized as a risk group. They may ingest daily large amounts of nitrate in drinking water and they are also more susceptible than adults to the adverse effects of nitrate, because relatively more nitrate is reduced to nitrite and the formation of methaemoglobin is induced at lower doses.

Adequate data on semichronic or chronic exposure of humans to nitrite are not available. The no-toxic effect level of 10 mg sodium nitrite.kg⁻¹ body wt, or 6.7 mg NO₂⁻.kg⁻¹ body wt, was derived from a long-term toxicity study in rats, the nitrite being administered via drinking water. In this study, the most sensitive parameter, the formation of methaemoglobin, was observed from the second lowest dose of 100 mg sodium nitrite.kg⁻¹ body wt onwards. Nitrite is mutagenic and caused morphological changes in in vitro systems. Mutagenic activity was also found in a combined in vivo-in vitro experiment with Syrian hamsters. In vivo experiments gave conflicting results. The mutagenesis observed in some in vivo experiments is probably due to deamination, while in other in vivo studies endogenous formation of nitroso compounds may play a role.

Although a few studies with nitrite showed a slight increase in tumour incidences, probably due to the nitrosamines present in the diet of the mice and rats, the final conclusion is that no clear evidence exists that nitrite itself is carcinogenic.

On the realistic assumption that in adults 5% of the nitrate is converted to nitrite, the no-toxic effect level of nitrate can then be derived from that of nitrite in rats:

$100\%/5\% \times 10 \text{ mg.kg}^{-1} \text{ body wt} = 200 \text{ mg sodium nitrate.kg}^{-1} \text{ body wt}$, or $146 \text{ mg NO}_3^-. \text{kg}^{-1} \text{ body wt}$. The average daily intake of adults is estimated at 110

mg NO_3^- , or $1.83 \text{ mg NO}_3^-. \text{kg}^{-1}$ body wt, so that there is a safety margin of a factor of 80 between the actual consumption and the no-toxic effect level. The bacterial reduction of nitrate to nitrite in children up to 3 months of age can be much higher than in adults. Assuming that it is 80% (Corré and Breimer, 1979), the no-toxic effect level for nitrate, derived from nitrite, which can be used for extrapolation to young children is then $100\%/80\% \times 10 \text{ mg.kg}^{-1} \text{ body wt} = 125 \text{ mg sodium nitrate.kg}^{-1} \text{ body wt}$, or $9.1 \text{ mg NO}_3^-. \text{kg}^{-1} \text{ body wt}$. Furthermore, infants under the age of 3 months are more susceptible to methaemoglobin formation than adults, which means that the actual no-effect level applicable to children may be even lower. The daily consumption of drinking water by bottle-fed infants is on average 500 ml in the first few weeks of life, increasing to around 850 ml in the second and third months (Nelson, 1983). According to the current standard, the upper limit permitted for nitrates in drinking water is $50 \text{ mg NO}_3^-. \text{l}^{-1}$. The actual ingestion of NO_3^- by children weighing from 3 to 5 kg can be calculated from these data to be from 8.3 to 8.5 $\text{mg.kg}^{-1} \text{ body wt per day}$. This implies that for bottle-fed infants it is virtually impossible to indicate a margin between the amount ingested and the no-toxic effect level with long-term exposure.

However, a few relevant remarks should be made here.

1. Several authors accept an increased methaemoglobin level of 10 % and over as the criterion for acute methaemoglobinaemia in infants (e.g. Winton et al., 1971). Cyanosis occurs at this level. In Shuval and Gruener's study on rats (1972), the methaemoglobin level observed with a dose of $100 \text{ mg NO}_2^-. \text{kg}^{-1} \text{ body wt}$ rose to less than 10 % (viz. 5 %) of the total haemoglobin. Extrapolated to infants this would mean that no (clinical) methaemoglobinaemia would occur at $91 \text{ mg NO}_3^-. \text{kg}^{-1} \text{ body wt}$. The margin between intake and this so-called marginal- effect level for infants is a factor of 11. For comparison: to calculate an acceptable daily intake (ADI) from animal experiments, it is customary to apply a safety factor between intake and the no-toxic effect level established in chronic animal studies. It consists of a factor to compensate for the uncertainties associated with extrapolation from animals to humans (usually 10), and a factor to compensate for differences in individual sensitivity (usually also 10); in this case, however, it already concerns the most sensitive group, so that only uncertainties in the extrapolation from animals to man need to be taken into account.

2. Clinical cases of methaemoglobinaemia in infants (cyanosis) were observed in practice almost exclusively in areas where the drinking water contained $> 10\text{-}20 \text{ mg NO}_3^- \cdot \text{N.l}^{-1}$ (which equals $> 44.3\text{-}88.6 \text{ mg NO}_3^- \cdot \text{l}^{-1}$; WHO, 1984; 1985).

With reference to cases of infantile methaemoglobinaemia, some authors have suggested that endogenous synthesis of nitrate and nitrite would be the main causative factor of methaemoglobinaemia in infants due to gastrointestinal infections (e.g. Hegesh and Shiloah, 1982). Increased reduction of nitrate to nitrite is indeed observed with some infections, sometimes - but not necessarily always - in combination with sharply increased endogenous synthesis of nitrate. This is however only one of the possible causes of methaemoglobinaemia. The results of Cornblatt and Hartmann (1948) confirm that the findings from animal studies (where oral intake of nitrate led to elevated haemoglobin levels) apply to children too. True, the dosage in this study was high, and no cyanosis developed, but methaemoglobin levels rose to 7.5 % of the total haemoglobin while exposure was only short and most children were moreover older than the "critical" age of 3 months.

Calculation of the quantitative risks of longer-term exposure can only be based on animal experiments, as described in detail above. Not only the possibly increased rate of endogenous nitrate synthesis but especially also the enhanced reduction of nitrate to nitrite in infants with gastrointestinal infections are a further reason for limiting as far as possible exogenous nitrate intake in this sensitive group.

The average daily consumption of nitrite by man is estimated to be 2.3 mg NO_2^- , or $38 \mu\text{g.kg}^{-1}$ body wt, so that the margin of safety for nitrite between intake and no-toxic effect level is a factor of 176, not taking into account endogenous nitrite formation. Unlike the situation with nitrate, there is no increased intake of nitrite by young children, because food with relatively high nitrite levels consists chiefly of meat products. As has already been mentioned, young children are particularly susceptible to methaemoglobin formation.

As stated elsewhere, the toxic activity of nitrate is determined not only by its conversion to nitrite but also by the endogenous formation of carcinogenic nitroso compounds (nitrosamines) from nitrite and nitrosatable compounds. In assessing the maximum cancer risk for lifelong ingestion of nitrate and nitrite, and products of endogenous nitrosation, many uncertainties have to be taken into account. Not only must nitrate and

nitrite be present but nitrosatable compounds, such as amines, must be available concomitantly in sufficient amounts. Endogenous nitrosation has been demonstrated in studies on human volunteers who consumed a variety of nitrate-containing food. These studies found traces of volatile nitrosamines (dimethylnitrosamine and diethylnitrosamine) when vegetables rich in nitrate and fish were consumed simultaneously. No or negligible nitrosation was found when vegetables together with meat were consumed, at least insofar that detection methods for nitrosamines were available (viz. volatile nitrosamines).

The rate at which nitroso compounds are formed under acid conditions varies for the different amines and amides, and not all possibilities of nitrosation have been investigated. Since endogenous formation of dimethylnitrosamine and diethylnitrosamine has been demonstrated in humans, the acceptable daily intake of these two nitrosamines for man (Dm), with an acceptable cancer incidence of $1:10^6$, is calculated on the basis of the most conservative one-hit model with linear extrapolation. These Dm values are:

- nitrosodimethylamine: $16-186 \times 10^{-6}$ mg (based on liver tumours),
- nitrosodiethylamine: $11-14 \times 10^{-6}$ mg (based on liver tumours) or $7.5-13.5 \times 10^{-6}$ mg (based on tumours of liver and oesophagus).

Based on average consumption of nitrate and nitrite in the Netherlands of 110 mg NO_3^- per person per day and 2.3 mg NO_2^- per person per day respectively, the possible endogenous nitrosation of a range of nitrosatable compounds can be calculated using the nitrosation figures from the study by Oshima and Bartsch (1981), who determined the amount of N-nitrosoproline in the urine of humans after ingestion of both nitrate and proline. N-nitrosoproline is for the most part excreted unchanged into the urine. They found that after ingestion of 95 mg or 130 mg nitrate together with 500 mg proline, the formation of N-nitrosoproline was 3 and 2 μg respectively. When 65 mg nitrate together with 500 mg proline was ingested, the formation of N-nitrosoproline was hardly detectable. It should be pointed out here that dimethylamine and diethylamine are nitrosated less rapidly than proline while morpholine and pyrrolidine are nitrosated more rapidly than proline.

With an average intake of 110 mg NO_3^- per person per day, it can be assumed, based on the data of Oshima and Bartsch (1981), that around 2 μg nitrosamines can be formed per person per day.

With an average intake of 2.3 mg NO_2^- per person per day, it can be calculated from the 5% conversion of nitrate to nitrite that this amount of nitrite could have been produced from $110\%/5\% \times 2.3 \text{ mg} = 46 \text{ mg } \text{NO}_3^-$. According to Oshima and Bartsch (1981) no measurable amounts of nitrosamines are formed from this amount of NO_3^- .

The estimated intake of preformed nitrosamines via food is 0.6 to 1.4 μg per person per day, thus the total daily exposure to nitrosamines can amount to 2.6 to 3.4 μg per person. The maximum cancer risk, based on the induction of liver tumours (in rats by diethylnitrosamine), is 9×10^{-5} with daily exposure to 1 μg of exogenous nitrosamines for a lifetime, but this risk becomes $3 \times 9 \times 10^{-5} = 27 \times 10^{-5}$ when nitrosation of ingested nitrate in the body is included. The above risk assessment is based on two conservative assumptions, namely

- 1) a relatively high endogenous nitrosation, based on the nitrosation of proline derived from the study by Oshima and Bartsch (1981).
- 2) the application of a "one-hit" model with linear extrapolation in the calculation of the maximum cancer risk for man from animal data.

However, it is difficult, if not impossible, to demonstrate in epidemiological studies a cause-effect relationship between low-level exposure to nitrosamines and the incidence of cancer in the general population. Data from a number of epidemiological studies are merely suggestive.

Epidemiological research in persons with stomach lesions or diseases shows that a correlation between the induction of gastric cancer and the intake of relatively large amounts of nitrate cannot be ruled out in this group. No link between the estimated nitrate intake and the incidence of stomach cancer could be demonstrated in persons without stomach lesions or complaints. Increased tumour incidences were not observed in persons occupationally exposed to nitrate or nitrite.

5.4.2. Ecotoxicity

Aquatic organisms

Given the objective of this document, only the data on nitrate have been evaluated.

Because of the difference found in toxicity between NaNO_3 and KNO_3 (based on nitrate content) in short-term toxicity tests on freshwater organisms, the available short-term tests with these two substances have been

evaluated separately. Data on KNO_3 for long-term tests in freshwater do not exist and they are inadequate in the case of both short- and long-term tests in seawater; only the data on NaNO_3 could be evaluated in these instances.

Generally accepted methods for extrapolating the results of laboratory tests (where usually only one species is tested) to natural ecosystems are currently not available. Provisionally, therefore, two different theoretical methods will be used for calculating "safe" levels for the aquatic environment. This approach also makes it possible to test the utility of these methods.

The method of Slooff et al. (1986) uses one toxicity level, either an L(E)C50 value obtained in a short-term test or an NOEC value from a long-term test. The value chosen is in both cases the lowest value available which, on the basis of the conduct of the test and from the results of the other tests, is considered to be sufficiently significant. Kooijman's method (1985, 1987) uses either all available L(E)C50 values or all available NOEC values. Van Straalen's method (1986), which was derived from that of Kooijman (1987), uses all available NOEC values. The reader is referred to the original publications for further details about these methods.

The results of the two extrapolation methods are summarized in table 5.1.

Table 5.1. Derivation of "safe" values for nitrate (in $\text{mg NO}_3^- \cdot \text{l}^{-1}$) in the aquatic environment according to Kooijman (1985, 1987), Slooff et al. (1986) and Van Straalen (1986)

		freshwater	seawater
<u>Slooff et al.</u>			
<u>NaNO₃</u>			
I.	<u>L(E)C50 (short-term)</u>	<u>2614</u>	<u>2700</u>
I.	--> calculated NOEC UF = 25.6 --->	65.53 2.56	67.58 2.64
	ss "safe"		
I.	--> calculated NOEC UF = 85.7 --->	44.46 0.52	45.65 0.53
	eco "safe"		
II.	<u>NOEC (long-term)</u>	<u>5</u>	-
II.	--> calculated NOEC UF = 33.5 --->	5.9 0.18	
	eco "safe"		

Table 5.1. continued		freshwater	seawater
KNO₃			
I.	<u>L(E)C50 (short-term)</u>	<u>229</u>	-
I.	--> calculated NOEC UF = 25.6 --->	8.35 ss "safe" 0.33	
I.	--> calculated NOEC UF = 85.7 --->	7.68 eco "safe" 0.09	
II.	<u>NOEC (long-term)</u>	-	-
Kooijman			
NaNO₃			
I.	<u>L(E)C50 values</u>	+	+
I.	--> "safe" L(E)C50 value [@]	121	147 (n = 1000 [*])
II.	<u>NOEC values</u>	+	-
II.	-->	"safe" 0.12 (n = 1000 [*]) 0.36 (n = 100 [*]) 1.05 (n = 10 [*])	
KNO₃			
I.	<u>L(E)C50 values</u>	+	-
	--> "safe" L(E)C50 [@]	0.30 (n = 1000 [*])	
II.	<u>NOEC values</u>	-	-
Van Straalen			
NaNO₃			
II.	<u>NOEC values</u>	+	-
	-->	"safe" 1.78	
KNO₃			
II.	<u>NOEC VALUES</u>	-	-

ss) "single species"; eco) "ecosystem"; UF) "Uncertainty Factor"

-) no data available; +) data available

@ "safe" L(E)C50: calculated value for the most sensitive species

* extrapolated to the number of species indicated

L(E)C50: "Lethal (effective) concentration for 50% of the exposed organisms"

NOEC: "NO-observed-effect concentration"

Final evaluation of nitrate in surface water

This final evaluation is based on 1) the results of the available toxicity tests and the above-mentioned extrapolation of these results to natural ecosystems, and 2) the section dealing with eutrophication (see section 5.3.1. and table 5.1.).

Freshwater

Based on the data concerning eutrophication, the evaluation of freshwater has been divided into "oligotrophic water" (fens and other surface waters not fed by rivers and "eutrophic water" (rivers and surface waters fed by them).

Oligotrophic water bodies

From the eutrophication point of view, a maximum concentration of $0.04 \text{ mg NO}_3^- \cdot \text{l}^{-1}$ has been recommended for oligotrophic water. The "safe" concentrations calculated from the results of the toxicity tests are all higher than this concentration. Based on this, the concentration of $0.04 \text{ mg NO}_3^- \cdot \text{l}^{-1}$ is also considered to be safe for both individual species and ecosystems at long-term exposure. This concentration has therefore been proposed as the maximum acceptable concentration in oligotrophic waters.

Eutrophic water bodies

On the basis of the section on eutrophication, the above-mentioned concentration of $0.04 \text{ mg NO}_3^- \cdot \text{l}^{-1}$ is considered to be unrealistic for the greater part of the Dutch surface water, either at present or in the future, so that a different concentration has been proposed for these water bodies. From the eutrophication viewpoint, a concentration of $1.8 \text{ mg NO}_3^- \cdot \text{l}^{-1}$ has been recommended for rivers and surface waters fed by rivers. From the results of the toxicity tests, "safe" concentrations have been calculated varying from $0.09\text{-}0.33 \text{ mg NO}_3^- \cdot \text{l}^{-1}$ based on tests with potassium nitrate, and $0.12\text{-}2.56 \text{ mg NO}_3^- \cdot \text{l}^{-1}$ based on tests with sodium nitrate. Most of these concentrations are considered to be unrealistic as the maximum acceptable concentration for eutrophic waters. In view of the large degree of uncertainty in the extrapolation methods used, a concentration of $2 \text{ mg NO}_3^- \cdot \text{l}^{-1}$, based on all available data, have therefore provisionally been proposed as the maximum acceptable concentration in eutrophic waters. It should be noted here that this concentration is not much lower than the only available NOEC value of $5 \text{ mg NO}_3^- \cdot \text{l}^{-1}$, derived from a study on early developmental stages of a few species of salmonid fish

Seawater

As regards eutrophication, $0.1 \text{ mg N} \cdot \text{l}^{-1}$ has been recommended for the total amount of nitrogen nutrients (NO_3^- and NH_4^+). This corresponds to about $0.4 \text{ mg NO}_3^- \cdot \text{l}^{-1}$ if all this nitrogen is present in the form of nitrate. The

"safe" concentrations calculated according to Slooff et al. (1986) from acute toxicity data (chronic data are not available) as 2.64 and 0.53 mg $\text{NO}_3^- \cdot \text{l}^{-1}$ for single species and ecosystems respectively. Based on these data, 0.4 mg $\text{NO}_3^- \cdot \text{l}^{-1}$ has provisionally been proposed as the maximum acceptable concentration in seawater.

Terrestrial ecosystems

Few data are available on direct toxic effects of nitrate on terrestrial ecosystems. In view of the rapid conversion of nitrogen compounds in the soil, it is preferable to work with total nitrogen rather than nitrate. Special attention is therefore paid here to the effects on terrestrial ecosystems of eutrophication due to nitrogen deposition.

Section 5.3.5. related the deposition, for four types of ecosystem, to the fluxes of nitrogen in undisturbed systems. In some instances it was already almost impossible to obtain data on undisturbed systems (heath). The effects from eutrophication can be brought about in part by the occurrence of soil acidification. This is especially true of naturally nutrient-deficient soils. Interaction of various effects, e.g. acidification leading to mobilization of toxic substances, hampers the analysis of effects caused exclusively by nitrogen.

Effects on forest ecosystems occur at N loads of 20-30 kg $\text{N} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$ and over, whereby such factors as age and productivity should be taken into account. No allowance has been made for the contribution of sources other than deposition.

Heath grasses up at N loads upwards of 14 (dry heath) and 20 (moist heath) kg $\text{N} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$, whereby effective nitrogen management remains essential.

For grasslands, a fairly well-founded value can only be given for effects on soil fauna and entomofauna: 50 kg $\text{N} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$ which, however, differs markedly for each species.

Quantitative data on dune ecosystems are insufficient for the calculation of an acceptable nitrogen burden.

Based on the foregoing, a deposition of 5-10 kg $\text{N} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$ has been proposed as a "safe" value for forest ecosystems. This value may be somewhat higher for deciduous than for coniferous forests. This value also provides a reasonable degree of protection for other terrestrial ecosystems.

6. MEASURING TECHNIQUES AND MEASURING STRATEGIES

6.1. MEASURING TECHNIQUES

6.1.1. Introduction

The last step in nearly all methods for measuring nitrate in the different environmental compartments is the determination of the nitrate ion dissolved in water. Reduction of nitrate to nitrite, which is subsequently determined photometrically, finds the widest application, especially in automated form. In addition, liquid chromatographic techniques have been gaining ground in recent years. Ion chromatography is often chosen because it enables the simultaneous determination of several anions.

An important recent publication is the report of the "Symposium on Anorganic Anion Analysis", held in Regensburg (FRG) from 19 to 21 September 1984 (Heumann, 1985).

6.1.2. Determination of the nitrate ion, dissolved in water

Spectrophotometric measuring techniques

In almost all photometric methods, nitrite is included in the measurement or it interferes with the determination of nitrate.

It must first be removed, or a correction must be made for it.

An major group of measuring methods is based on the reaction of nitrate with sulphuric acid and a substituted phenol with the formation of a nitro compound. Salicylic acid is used in the German "Trinkwasserverordnung" (Wagner, 1979) and the Dutch NEN 6440 standard (1981), and has a lower detection limit of around $10 \mu\text{g NO}_3^- \cdot \text{N} \cdot \text{l}^{-1}$. Chromotropic acid is the specific reagent used in an American standard method (Standard Methods, 1981), a refinement of which has also been described (Bachhausen et al., 1985). Xylenol and 4-fluorophenol are used in the standard specifications DIN 38405, D9 (1979) and ISO 7890/1 or ISO 7890/2, with 4-fluorophenol working better in strongly coloured water owing to the application here of steam distillation and/or extraction. Automated versions have been described by Wagner et al. (1979) and Hoppner et al. (1979).

Determination of nitrate in seawater, via an oxidation reaction with brucine or strychnidine, is being carried out with varying results (see Ranney and Bartlett for a rapid field method, 1972). The determination via reduction to ammonia or hydroxylamine is hardly ever used ((Standard

Methods, 1981; Bajic and Jaselskis, 1985). Most widely used is estimation of nitrate after its reduction to nitrite. Copper-cadmium alloy usually acts as the reducer (see Nydahl (1976) for the mode of operation, Hydes and Hill (1985) for an improved design). Following reduction, the resulting nitrite is determined by a standard photometric method: reaction with sulphanilamide in acidic medium, yielding a diazo compound, which is then coupled to N-1-naphthylenediamine with the formation of a red colour. An "Auto-Analyzer" version of this method is included in the "Standard Methods" (1981). The method is suitable for freshwater and seawater and has a lower detection limit of about $2 \mu\text{g NO}_3^- \cdot \text{N.l}^{-1}$. A drawback is that the measurement obtained includes nitrite so that either a difference determination must be made or nitrite must first be removed (for interferences, see also Norwitz and Keliher, 1985). There is also a flow-injection variant (Nakashima et al., 1984; Gine et al., 1983; Johnson and Petty, 1983). In view of the carcinogenicity of the colour reagents, alternatives are being researched (see e.g. Werner, 1983).

Nitrate dissolved in water can also be determined by direct measurement of its extinction in the ultraviolet spectrum. Other UV-absorbing substances, such as nitrite and organic compounds, should either be absent or removed, for example, with activated carbon or by dialysis (especially in the case of humic matter). This method is used in the Dutch standard for rainwater (NEN 6581, 1985), with a lower detection limit of around $15 \mu\text{g NO}_3^- \cdot \text{N.l}^{-1}$, and it is also included in the American "Standard Methods" (1981).

Several manufacturers have introduced kits into the market with which, for example, nitrate in water can be determined semi-quantitatively in the field. They all make use of a colour reaction. Schwedt and Rienaecker (1983) compared a number of such kits with assays using the German standard methods, for nitrate DIN 38405-D9.

The determination of nitrate by polarography is not much used (Merz et al., 1978). The application of ion-selective electrodes is limited because of interferences by other anions, such as chloride and bicarbonate, although it has been reported (Standards Methods, 1981; Fayad et al., 1979; Eicken, 1977; Hara et al., 1985; Orion Research, 1981; Camman, 1977).

In recent years, increasing use is being made of HPLC for the determination of nitrate (and nitrite) in unpurified extracts of soil, plants, foodstuffs and the like (section 6.1.6.). In general, the absorption of UV by both anions is used for detection. Thayer and Huffaker (1980) investigated HPLC determination of nitrate and nitrite. They used an anion-exchange column and a phosphate buffer (pH 3) as the elutant. They were able to measure as little as 3 ng NO_3^- and 9 ng NO_2^- in samples of from 5 to $500 \mu\text{l}$.

Variants have been described by Alawi (1982), Iskandarani et al. (1982), Kok et al. (1983), Mulling et al. (1984), de Kleyn and Hoven (1984), Jackson et al. (1984) and Kuchnicki et al. (1985).

Another form of liquid chromatography, ion chromatography, also finds wide application for the determination of nitrate, especially in water. The great strength of ion chromatography lies in its ability to determine simultaneously a large number of anions side-by-side, if required, automatically. In addition, the samples can be concentrated "on line" (Okada et al., 1985), thereby making possible the lowering of the detection limit from around $50-100 \mu\text{g.l}^{-1}$ to around $1 \mu\text{g.l}^{-1}$. This method is not suitable for seawater because of its very high chloride level ($19,000 \text{ mg.l}^{-1}$).

Ion chromatography is used for the analysis of drinking water and groundwater, but especially for rainwater for which a draft standard is available with a lower detection limit of about $30 \mu\text{g NO}_3^- \cdot \text{N.l}^{-1}$ (NEN 6588). The following publications give a good introduction to the literature: Slanina et al. (1981), Schwabe et al. (1983), Darimont et al. (1983), Resch and Gruenschlaeger (1984), Dits and Smeenk (1985), Colenutt and Trenchard (1985), and the papers presented at the above-mentioned symposium on anion analysis.

Finally, nitrate can be determined by gas chromatography. It is relatively simple to convert nitrate in a sulphuric acid medium into nitrobenzene, nitrotoluene or a nitrophenol. The reaction product obtained can be determined by gas chromatography with high sensitivity when an electron-capture detector is employed. The smallest amount which can thus be measured is about 1 pg NO_3^- . In absolute terms, this is therefore the most sensitive nitrate assay known. There are few interferences and the method is widely used especially for the determination of nitrate in small volumes of biological material. The gas-chromatographic part of the assay can be automated, but not yet the derivatization procedure. Relevant publications are those by Ross et al. (1975), Tesch et al. (1976), Wu et al. (1977), Toyoda et al. (1978) and Tanaka et al. (1982).

6.1.3. Determination of nitrate in soil and sediment

In general, the soil is extracted and then analyzed by ion chromatography or photometry. Kuchnicki et al. (1985) found that extraction with a methanol-water mixture gave a 19% higher yield of nitrate and nitrite than extraction with pure water.

Wilken and Kok (1985) have described the determination of nitrate, among other substances, in the pore water of sediments from the Elbe estuary. Samples of the cut-out sediment are centrifuged, yielding a small volume of brown-coloured water for analysis. When the electric conductivity of this water is less than 1 ms.cm^{-1} , i.e. the sample contains little chloride, nitrate can be measured with a nitrate ion-selective electrode. Ion chromatography is employed when the conductivity is more than 1 ms.cm^{-1} .

6.1.4. Determination of nitrate in various types of water

Ion chromatography and photometry are the most widely used methods for rainwater and drinking water. A photometric determination is the preferred method for surface water. For brackish water and seawater, reduction to nitrite is used almost exclusively, usually in Auto-Analyzer form. When this method has become operational, it will also be used for other types of water. Allowance should be made here for the influence of the salt level on the colour formation. The nitrate concentrations relevant to oceanography are very low, in the low $1 \mu\text{g.l}^{-1}$ range.

In many instances the water sample will be filtered prior to its analysis. However, it was found that many filter materials can release nitrate into the water. This is especially important when the nitrate concentrations to be measured are low (Jay and Judd, 1985; Jay, 1985; Bagchi and Haddad, 1986).

Ewald et al. (1985) have described the decolouration of strongly coloured water samples prior to photometric determination of nitrate. The resins employed were of the macroreticular type, without functioning groups, but, unfortunately, they were not further specified.

The literature gives many different directions for storing samples in such a way that the nitrate concentration does not change. Storage at -25°C seems adequate when the samples are stored for a few weeks. Both glass and polyethylene bottles can be used. The most important consideration is the prevention of any (micro)biological activity in the samples. They should be kept in the dark to prevent algal growth. In the presence of oxygen, bacteria can convert nitrite, and possibly also ammonium and organic nitrogen, into nitrate; heavily contaminated samples can rapidly become anaerobic, whereupon nitrate can be broken down. Some authors lowered the pH of the samples with acid. Others added chloroform or a mercury salt. The latter is not possible if a cadmium reductor has to be employed.

Relevant publications are: Wagner (1976), Sprenger (1978), Van der Vlies et al. (1978), MacDonald et al. (1982), Harmsen et al. (1983), Ridder et al. (1985) and El-Shaarawi et al. (1984).

The sampling of drinking water or surface water does not create any particular problems. For the sampling of groundwater, consult the Provisional Practical Guidelines of the Directorate General for Environmental Protection, published in 1986.

The correct collection of precipitation samples is a separate problem. See, for example, the publications by Slanina et al. (1979), Aichinger (1980) and Lewin and Torp (1982).

6.1.5. Determination of nitrate in the atmosphere

Nitrate can be present in the atmosphere as gaseous nitric acid and as nitrate particles, chiefly ammonium nitrate. Taking samples with filters does not yield accurate results because of interaction between the trapped particles and gases flowing past them. To circumvent this problem, the so-called filterpack method can be employed for the determination of total nitrate in air (Fugelsang, 1986). Another possibility is the use of a so-called denuder (Slanina et al., 1981). In this method the air to be sampled flows through a tube. The gas molecules can reach the wall of the tube by diffusion and are absorbed here. The aerosol particles in the laminar gas flow cannot reach this wall and are collected on a filter after their passage through the tube. The surface of the denuder is coated with, for example, a mixture of glycerol and sodium carbonate. After taking the sample, filter and denuder are extracted with water, and nitrate in the extracts is determined by a photometric method or by ion chromatography. Other relevant publications are: Possanzini et al. (1983), Allegrini et al. (1984), Cadle et al. (1985), de Santis et al. (1985), Appel et al. (1981) and Shaw Jr. et al. (1982).

6.1.6. Determination of nitrate in foodstuffs and other biota

The following products are tested for the presence of nitrate: leaf vegetables and other vegetable matter, meat products, baby food, dairy products (especially cheese, whey products and milk powder). The upper limits to the nitrate concentration set by the Food and Drugs Act (see section 1.2.4.) are high in comparison with the detection limits.

Blood and urine are also frequently tested for nitrate.

The determination requires:

- taking a representative sample;
- homogenizing the sample and extracting the nitrate;
- determining the nitrate in the extract.

Sampling

The nitrate is usually not distributed homogeneously throughout the batch and its concentration is not constant with time. The nitrate content in lettuce and endive differs from one head to another; moreover, the concentration falls considerably during storage, already at the greengrocer's). There can be a large difference in the concentration measured between vegetables sampled in the morning and in the afternoon from the same field. The nitrate level in meat products and cheese, too, is not constant: the nitrate concentration continues to fall during the fermentation process. This means that the sampling procedure (location, time, quantity) should be clearly defined; in addition, as soon as the sample has been collected, it should be stored in such a way that changes in the nitrate level are negligible, for example, by freeze-drying (vegetables) or deep-freezing, or by homogenizing and extracting the sample as soon as possible for further analysis.

Homogenization and extraction

It is important to homogenize the sampled material properly, especially with leaf vegetables. The nitrate concentration in the outer leaves of lettuce and endive can be roughly twice as high as that in the inner leaves, and the nitrate concentration in the leaf stalks of spinach can be much higher than that in the leaves. To obtain a representative picture of a batch, the General Decree (Food and Drugs Act) stipulates quartering heads of lettuce and endive along the longitudinal axis and mixing quarters from different heads to give a collective sample, which is then ground. For solid foodstuffs, extraction with (hot) water is virtually the only method employed for the isolation of nitrate. The aim usually is to determine nitrate and nitrite in the same extract. Both substances must thus be extracted quantitatively. This presents an additional problem for protein-rich products in particular, because reactive nitrite, apart from occurring as such, also occurs bound in a wide variety of compounds, for example, nitrosothiols and nitrosyl myoglobin. The addition of metal salts (Ag, Ba, Cd and Hg salts), acetone or alkali to the extraction medium releases nitrite from such compounds and, in addition to free nitrite, a portion at least of the chemically bound nitrite is also measured. Since nitrite decomposes in solutions with a pH of 5 or lower, weakly acidic buffer

solutions or alkaline solutions are often employed for the extraction procedure. During the extraction of complex products such as cured meats, cheese, milk powder, etc. with a hot watery solution, many other (organic) substances, apart from nitrate and nitrite, are also extracted, including proteins. These can interfere with the subsequent stages of the determination, and they must be removed from the extract. This is referred to as "clean-up" of the extract and many, mainly anorganic, substances have been proposed for this purpose in the literature. Examples are: zinc sulphate, borax, potassium aluminium sulphate, zinc acetate, potassium ferrocyanide, sodium hydroxide, aluminium oxide, iron salts, and often mixtures of these. A critical review of this problem has been published by Usher and Telling (1975). The most widely used substances, in standard methods of ISO, IDF, NNI and Food and Drugs Act among others, are at present the "Carrez reagents", solutions of zinc sulphate or zinc acetate and potassium ferrocyanide, which are added successively. Ascorbic acid and ascorbates (in meat products and vegetables) readily react with nitrite. As a result, the nitrate level found can be too low, because the nitrate is often reduced to nitrite prior to its measurement. This interference can be counteracted by oxidation with permanganate (Usher and Telling, 1975).

Determination in the extract

When a clear, watery extract of the foodstuff has finally been obtained, its nitrate content can be determined in various ways (see also section 6.1.2.). Most reference methods make use of a photometric technique after reduction of the nitrate to nitrite (see, for example, NEN 3453/3554), sometimes in an Auto-Analyzer version. In addition, attention has focused especially on rapid methods based on ion-exchange chromatography on reversed-phase columns, which in recent years have been used more and more with success (Jackson et al., 1984; Schmidt and Schwedt, 1984; De Kleijn and Hoven 1984; Ellen et al., 1985). They will probably be just as reliable in the short term as the current photometric reference methods.

6.1.7. Quality assurance

Heumann and Unger (1983) and Unger and Heumann (1985) described an independent mass-spectrophotometric method with which the determination of nitrate in water and foodstuffs, respectively, can be calibrated.

A German ring study, in which 30 laboratories participated, compared three photometric assay methods: the 2,6-xyleneol method, the p-fluorophenol method and the sodium salicylate method. In no case was it possible to

establish a methodic standard deviation based on variance analysis, because the dispersion of the results between laboratories was 2.5 to 10 times greater than that within one laboratory. Addition of saccharose to one of the samples resulted in 25 % too high values and greater scatter.

Satisfactory results were obtained in a British ring study in which 10 laboratories participated, all using an automated assay of nitrate + nitrite and nitrite according to the Cd-reduction method. The results were all within the ± 20 % range for a concentration of $0.1 \text{ mg NO}_3^- \cdot \text{N} \cdot \text{l}^{-1}$ in river water (Gardner, 1982).

Less satisfied were the participants in an American ring study into the determination of nitrate in forage (McMahon and Casper, 1984). The interlaboratory coefficient of variation for 16 laboratories for 4 samples was 36, 51, 13 and 55 % respectively. The requirement was 15 % at most. Different methods were used. Very poor results were also sometimes obtained with the cadmium-reduction method.

Symader et al. (1985) conducted a study into the changes with time in the measurement error occurring over a long period in the determination of nitrate, among others, in Rhine water. They sought the cause especially in changes in the samples during storage prior to their analysis. These changes are dependent on the (not constant) composition of the river water. Wilhelms and Funk (1985) compared the German standard methods for the determination of nitrate, among others substances, with an automated method. The latter was quicker and had a better reproducibility.

Grundahl and Keidig (1985) proposed a quality control programme for rainwater analysis. However, they were not able to distinguish between analytical errors and "peculiar phenomena in the precipitation's chemistry".

6.2. MEASURING STRATEGY

6.2.1. General

The primary objective of a measuring strategy is to create a framework within which policy measures can be prepared or taken, on the basis of, if possible standardized, measurements on the occurrence of a substance in the different environmental compartments, drinking water and foodstuffs. Alternatively, the effect of measures previously taken can be evaluated, for example, by means of trend analysis. An important secondary objective is to draw up a measurement plan of maximum efficiency with which results can be obtained with lowest possible expense and effort, while meeting certain minimum requirements as to accuracy and intercomparability. Accordingly, on the one hand, a measurement plan should be closely related to the scale (local, regional, national) on which knowledge about the occurrence of a substance in the various environmental compartments is desired. On the other hand, a measurement plan, depending on the immission pattern, can include elements aimed at specific (point) sources. Lastly, the collection of measurement data for research in a broader sense into processes and effects can be mentioned as a derived objective.

Knowledge about the behaviour and transport of a substance is indispensable when planning a measuring strategy. Insight is needed into the chemical, physical and biological behavioural parameters, so that equilibrium situations can also be incorporated in a model. The setting of priorities is essential when cost aspects play a part. Comparatively more attention will be paid here to the most vulnerable situations or environmental compartments, with a relatively high ratio between concentration and effect-level.

For a structured approach to a measurement programme to be developed, it is useful to draw up a flow diagram, where the various issues relevant to the setup are considered systematically.

Such a flow diagram is presented in the following sections for the various environmental compartments.

6.2.2. Measuring strategy for soil

Figure 6.1. shows a flow diagram aimed at the development of a general measuring strategy for the soil (Van der Berg and Beugelink, 1986). Central in the development of a measuring strategy for nitrate in the soil is knowledge about its behaviour and transport in the subsoil, related to the soil conditions and the geohydrological situation. Since nitrate is denitrified under anaerobic conditions, there is no point in sampling anaerobic groundwater and analyzing it for the presence of nitrate. This makes, for instance, measurements in the clay-peat regions in the west and north of the country meaningless. The same is true for deeper-lying anaerobic water-carrying systems in the sandy regions.

In those regions where nitrate can occur in the water, the presence or absence of nitrate is strongly determined by the source conditions and the flow of soil water and groundwater as a transport medium for nitrate. Knowledge about the geohydrological situation is therefore indispensable for measurements in groundwater. A sound interpretation of measurement data is not possible without knowledge of this situation. In particular, when there are local nitrate sources such as overfertilized tracts of land, septic tanks and leaking sewers, channels of heavily contaminated water can be found as a result of poor mixing in groundwater, surrounded by a quality of groundwater of different origin. Very old groundwater without nitrate is usually found in deep seepage areas, while nitrate may be present in shallow seepage areas. Raw-water analyses of groundwater pumping stations cannot be used by themselves for making statements about the nitrate level of the groundwater. This is due to the fact that the catchment area per well and for the entire pumping station may be undergoing change, whereby the origin of the water drawn can vary.

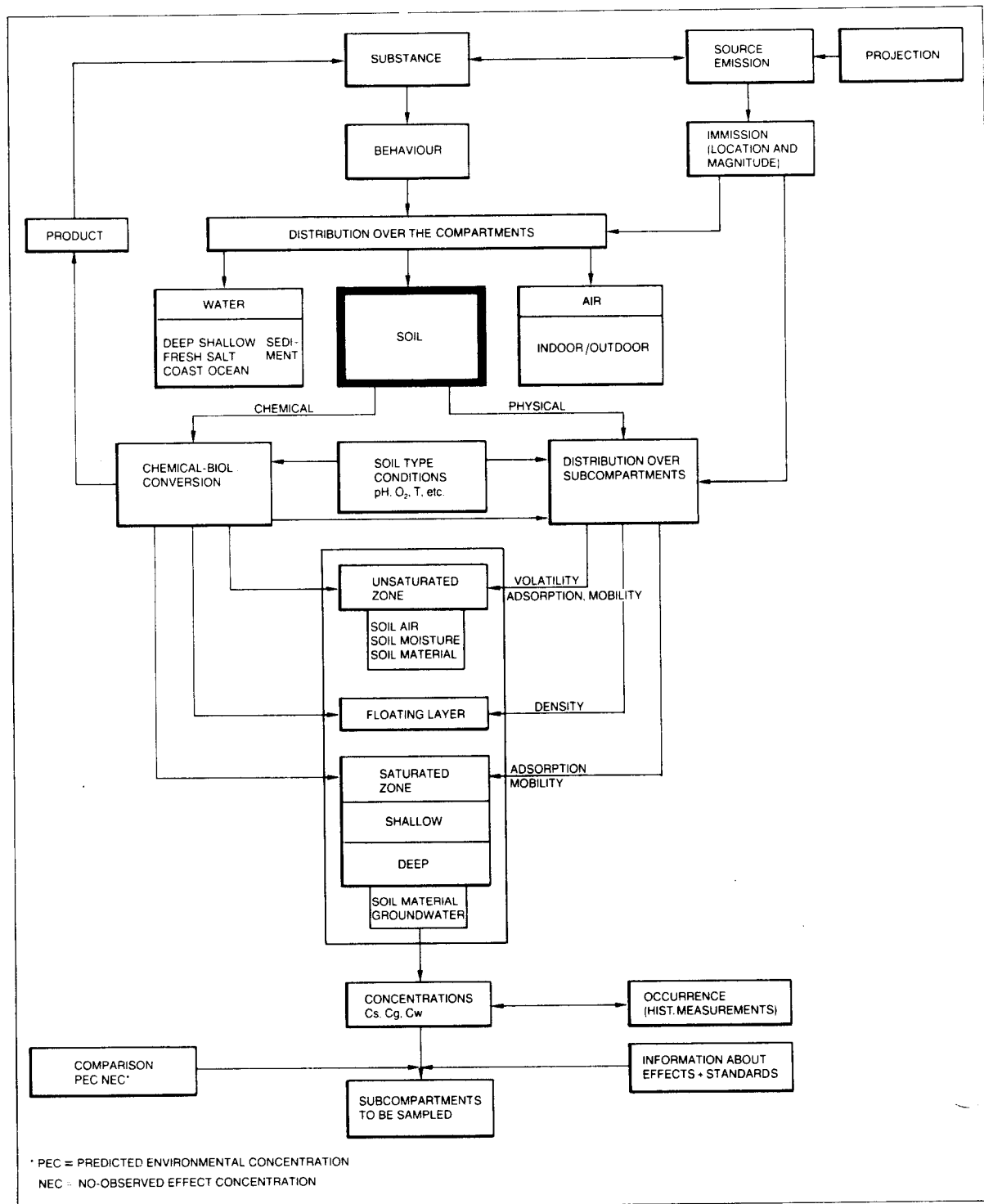


Figure 6.1. Flow diagram of measuring strategy for soil

6.2.3. Measuring strategy for surface water

When developing a measuring strategy for nitrate in surface water, it is all-important to realize that the nitrate ion is an intermediate in the nitrogen cycle. The nitrate concentration can alter as a result of changes in the input of nitrate as well as ammonium and organically bound nitrogen, and also by changes in conversion rates. The formation of nitrate from ammonium is heavily dependent on the temperature and the presence of oxygen. One of the principal controlling factors of changes in the nitrate level is the demand for nutrients by the autotrophic subsystem. In more or less stagnant water bodies (lakes, fens, ditches, canals and the like) the growth of vegetable matter (algae and water plants) is a much more important process than in running waters (streams and rivers). As a result, the nitrate concentration in stagnant water bodies exhibits a more pronounced seasonal rhythmicity (low concentrations in summer and high in winter) than that in running waters. The lower the degree of eutrophication of the waters to be tested, the more extreme will be the summer minimum in particular. To calculate roughly the amount of nitrogen potentially available for nitrate formation, it is recommended to perform ammonium, nitrite and organic nitrogen analyses in conjunction with nitrate measurements.

For the formation of a reliable picture which includes possible seasonal fluctuations in the nitrate concentration, sampling at least once a month is necessary. The efforts of the Department of Public Works (Large rivers, IJsselmeer, Wadden Sea, North Sea and Tidal waters) and the RIWA (Joint Rhine and Maas Water Companies; Large rivers and IJsselmeer), with a frequency of from once a week to once a month, amply meet these requirements. The impression exists that most regional water authorities, too, analyze the waters for which they are responsible at least once a month.

Figure 6.2. shows a flow diagram of a general measuring strategy for surface waters.

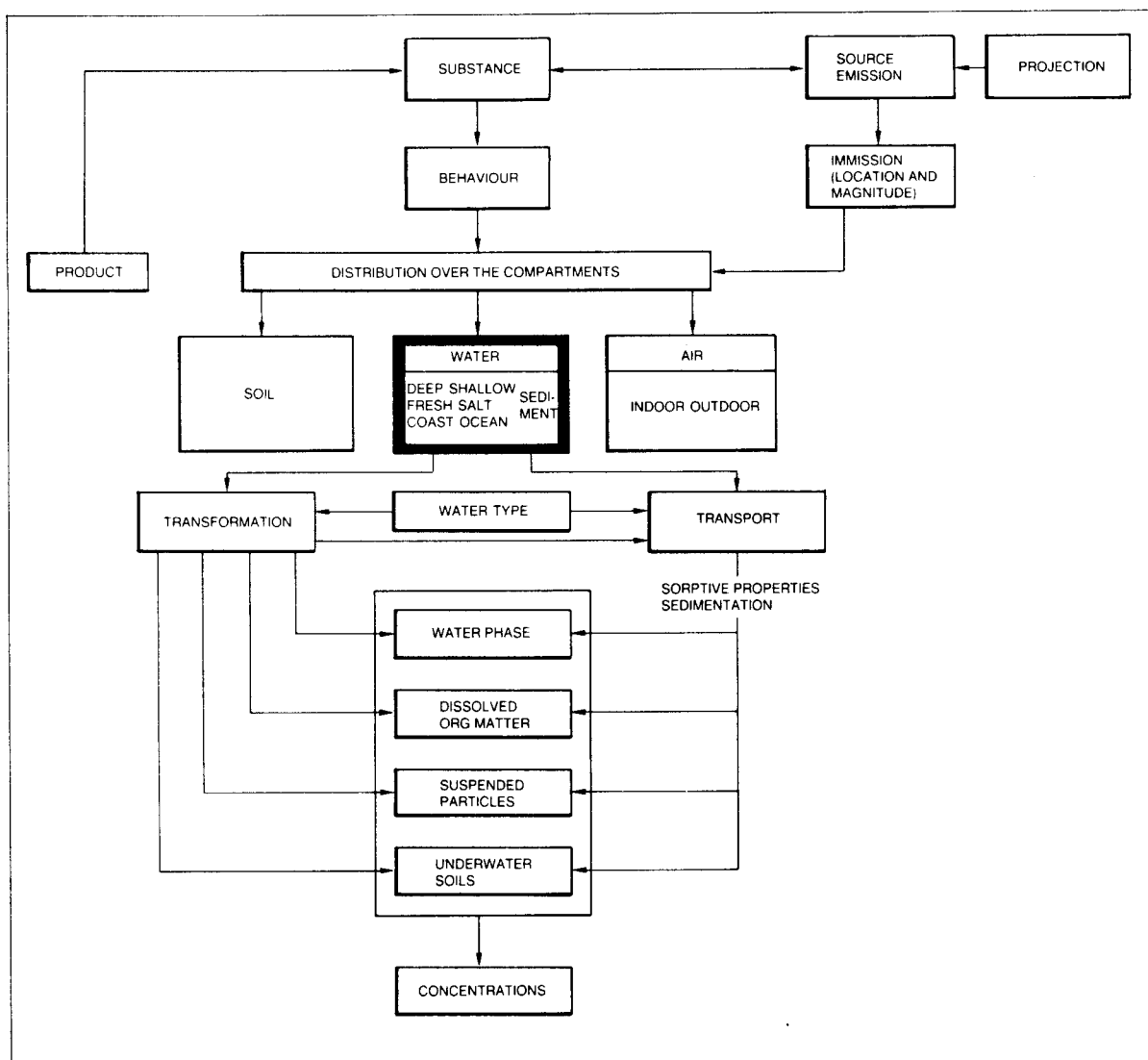


Figure 6.2. Flow diagram of measuring strategy for water

6.2.4. Measuring strategy for air

In drawing up the measuring strategy for the air, it was assumed that especially the determination of the deposition of nitrate (and other nitrogen compounds) onto the ground is important. In view of the advantages of model calculations (relatively low cost, much information about a large area, possibility of both origin and scenario calculations) and the current state of knowledge in this field, preference is given to a measuring strategy where measurements serve to support and validate model calculations.

Given the even distribution pattern of nitrate over the Netherlands (variation is generally less than 25 %, see ch. 4), about 6 measurement sites are sufficient for the determination of the wet depositions (measurement of nitrate in rainwater). For the calculation of the dry depositions, the concentration of gaseous and particulate nitrate is relevant. For the measurements, it is sufficient to determine the total nitrate in the air at about 6 stations, with a frequency of one 24-hour observation every 8th day at 4 of these stations.

Adequate information about the ratio of gaseous to particulate nitrate can be obtained from brief measuring campaigns and supplementary observations during an episode of increased air pollution.

Figure 6.3. shows a flow diagram of a general measuring strategy for air.

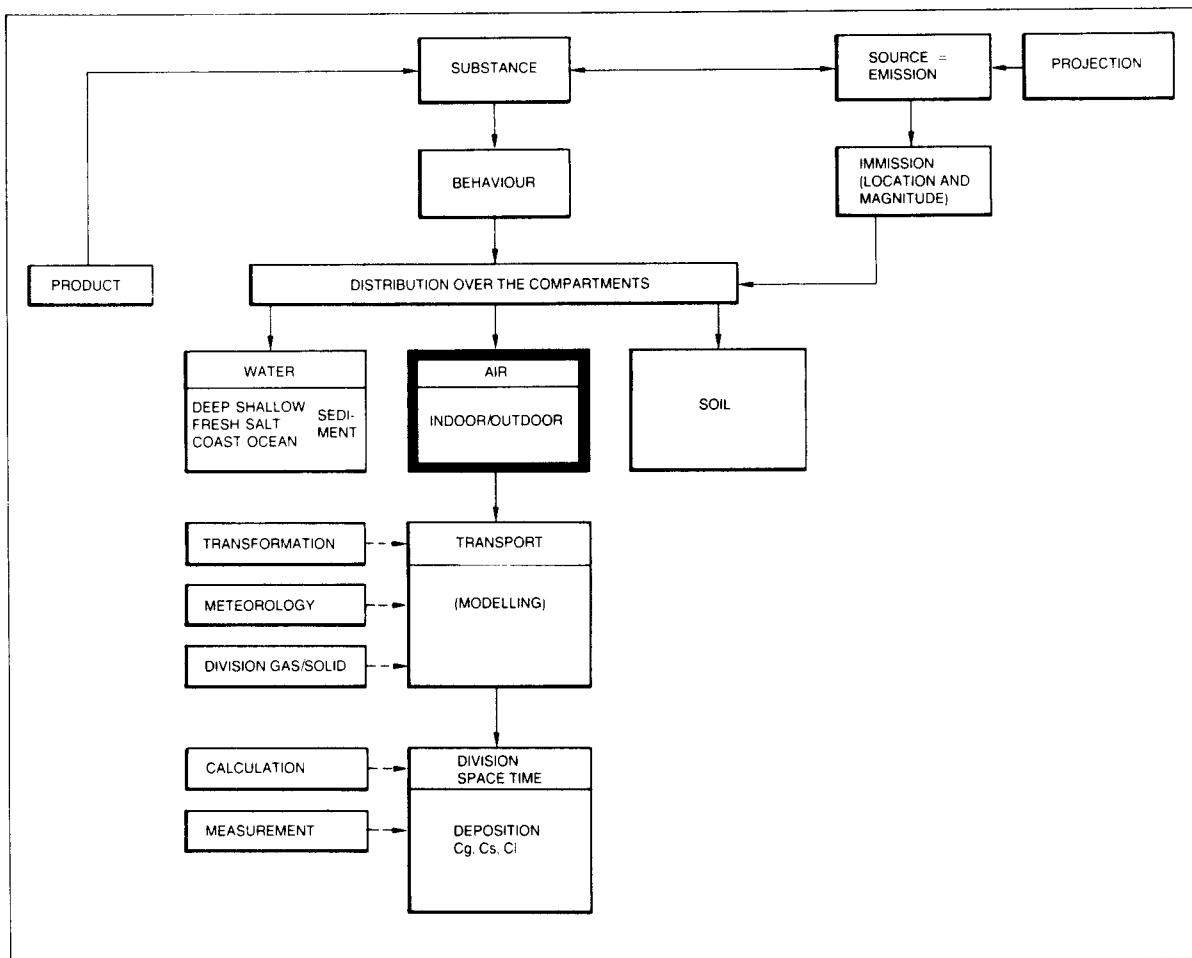


Figure 6.3. Flow diagram of measuring strategy for air

6.2.5. Measuring strategy for drinking water

The frequency of measuring nitrate in drinking water obtained from ground- and surface water is laid down by law in the Water Board Decree. Given the rate at which the nitrate concentration in ground- or drinking water is increasing, this frequency is sufficient to enable the water companies to take adequate measures against exceeding the standard. Data from the drinking water monitoring network (annual sampling at pumping stations by the RIVM by order of the HIMH (Chief Inspectorate of Environmental Protection)) are used in making a rough estimate of the future development in the nitrate concentration at all pumping stations (Van Dijk and Fonds, 1987). The national groundwater-quality monitoring network (RIVM) supplies data which, together with (geo)hydrological information and residence-time-dispersion models, give an indication of the time scale within which the nitrate-laden groundwater will reach the borehole supplies. The above provides an adequate safeguard for the public drinking water supply. However, a point demanding attention is the cataloguing of private water supplies.

More than 100,000 persons use drinking water from their own well. The nitrate concentration of this water often exceeds the standard considerably. This water is moreover also frequently used for watering livestock. Municipalities could in consultation with the water companies register the private water supplies. A measurement programme for determining the nitrate concentration in all wells (or a representative percentage thereof) could then be drawn up and implemented.

6.2.6. Measuring strategy for food

Vegetables, and leaf vegetables in particular, are by far the most important source of nitrate intake via food; this is roughly three-quarters of the total nitrate intake with the dietary pattern customary in the Netherlands. There are no reasons for assuming that the concentration of nitrate naturally present in other foodstuffs will increase substantially. Furthermore, the addition of nitrate and nitrite to foodstuffs (meat products and cheese) is controlled by regulations of the Food and Drugs Act. Statutory limits are in force for the nitrate content in lettuce, spinach and endive. The State Food Inspection Departments regularly analyze samples of these products for nitrate, thereby creating a good overview of the nitrate levels in these products and of the dispersion they show over a period of time. In view of the relatively high concentrations present in

these vegetables, their analysis does not present great problems; several (automated) methods are quite satisfactory at this level and, furthermore, the Food and Drugs Act describes a reference method which, although laborious, is reliable.

In addition to lettuce, spinach and endive, high nitrate levels are also found in other vegetables, such as beetroots, turnip tops and purslane. The levels in the two last-mentioned vegetables are often considerably higher than the limits in force for lettuce, spinach and endive. Although there are no statutory limits for the other vegetables, it is advisable that State Food Inspection Departments and agricultural institutes also sample and analyze regularly vegetables such as beetroots, turnip tops and purslane, so that a good overall picture is gained of the development in the nitrate concentrations of all nitrate-rich vegetables. There is not much point in regularly analyzing other foodstuffs because of the small contribution they make to the total nitrate intake. An exception is ready-to-eat baby and infant food, which has been tested regularly in connection with the vulnerability of this group to nitrate. To form a rough estimate of the actual average daily nitrate consumption, it is recommended to repeat periodically 24-hour duplicate portion analyses and market-basket studies, as performed by the RIVM and CIVO-TNO respectively.

6.3. Summary and conclusions

There are many methods for determining nitrate, for every chosen measuring range and for all environmental compartments. A distinction can be made between photometric, electrochemical and chromatographic techniques. In addition, there are various rapid field tests on the market. The nitrate-to-nitrite reduction and liquid chromatographic methods find the widest application. Some methods could still be improved, but research into the storage of samples and the organization ring studies have a higher priority. The automated methods are clearly preferred.

Knowledge about the behaviour and occurrence of nitrate and other nitrogen compounds in the various environmental compartments, drinking water and foodstuffs is indispensable for formulating a measuring strategy. Insight into the geohydrological situation and soil conditions is essential for measurements in the groundwater. In connection with the occurrence of seasonal fluctuations, a measuring frequency of at least once a month is considered advisable for surface water. For the environmental compartment air, a limited number of monitoring stations to support model calculations is sufficient. In the case of drinking water, not only the data from the

drinking water monitoring network are important but also data from the national groundwater-quality monitoring network. In addition, attention should be paid to private water supplies. Registration and regular sampling of these water supplies also deserves consideration. Vegetables are the major source of nitrate in foodstuffs. It is advisable that the State Food Inspection Departments regularly sample not only lettuce, spinach and endive, but also other vegetables like beetroots, turnip tops and purslane. Further special attention should be paid to ready-to-eat baby and infant food.

7. POSSIBILITIES AND COSTS OF EMISSION REDUCTION

7.1. SOIL

The emissions of nitrate and other nitrogen compounds to the soil have been described in chapter 2, namely:

- domestic and industrial waste water (incl. sludge), 14 ktonnes N per year;
- the use of chemical fertilizer, 111 ktonnes N per year;
- the use of animal manure, 270 ktonnes N per year;
- waste materials, 41 ktonnes N per year;
- spoil from dredging operations, 60 ktonnes N per year.

7.1.1. Domestic and industrial waste water

Nearly all the nitrogen present in domestic waste water is derived from human excrements. This implies that there are in fact no ways to prevent the nitrogen from entering waste water. The amount of nitrogen in industrial waste water could probably be reduced by modifications of processes and operation, but in view of the relatively small amount involved no attention will here be paid to this. The waste-water discharges from households into and onto the soil can be cut by connecting the dischargers to the sewerage system and transporting the waste water to a sewage treatment plant (rwzi), though this is only effective when the rwzi has been fitted with a nitrification-denitrification system (see section 7.2.), for the nitrogen compounds are then converted to N_2 . When the nitrogen compounds are not converted to N_2 in the rwzi, the diffuse emissions to the soil are merely changed to point discharges into the surface water. Emission reduction is directly dependent on the percentage of connections to the sewerage system, leakage from the sewers, rainwater overflows and the extent of denitrification in the sewage treatment plant. About 90% of the households in 1980 were connected to the sewerage system. This had risen to around 95% in 1986. The cost of connecting the last 5% can roughly be put at f 10,000.- per connection. Of the remaining 5%, a small fraction (1% at most) could possibly be connected for the highest acceptable sum of around f 15,000.- per connection, while the other 4% cannot be sewered. Of these 4%, about 40% (280,000 inhabitant equivalents) discharge into the ground. Several treatment systems are known for individual dischargers, but they have no influence on the nitrogen/nitrate level of the waste water.

7.1.2. Chemical fertilizer and animal manure

The use of chemical fertilizer has remained approximately constant over the past few years. The autonomous development in the volume of animal manure produced is regulated by various restrictive measures, such as the Restriction of Pig and Poultry Farming Interim Act (which was in force until January 1987), the Soil Protection Act, the Manures Act and the Ecology Guideline of the Nuisance Act. The superlevy introduced by the EEC also influences the number of cattle and thereby the volume of manure. In the first instance, the measures aim to stabilize manure production, and only in the later phases will reduction be possible. The total number of cattle has fallen between 1985 and 1986 from 5,516,243 to 5,122,950 head of cattle (a reduction of 7%). The size of the decrease makes it seem likely that it is the result of the superlevy. The number of pigs has risen between May 1984 and May 1986 from 11.1 million to 13.5 million pigs, an increase of 21%. The total number of poultry has increased from 83.4 million to 89.9 million chickens, a rise of about 8%. Measures to limit the emission of nitrogen compounds into the soil from the application of fertilizers are:

- replacing animal manure by chemical fertilizer. Since less leaching occurs with chemical fertilizer than with animal manure, this measure leads to decreased emissions to the soil (see ch. 2, fig. 2.1. and 2.2.). One condition is, however, that the volume of available animal manure falls proportionally, for example, as a result of reduced production or of manure processing;
- better adjustment of nitrogen fertilization to the plant requirements. For the time being, however, various problems have to be solved before simulation techniques developed for this purpose can be applied in practice (Neeteson, 1985);
- when fertilizing, taking into account the amount of soil N available in the spring;
- reducing the amount of nitrogen in the fodder (Jongbloed and Simons, 1985).

The leaching of excess nitrogen, which is not utilized in the agricultural production system, can be reduced as follows:

- leaving agricultural land covered in the autumn;
- employing nitrification inhibitors;
- a modified manure spreading regimen;
- increasing the moisture content in the water-unsaturated zone by, for example, raising the water table and/or sprinkling.

The use of nitrification inhibitors and alteration of the manure spreading regimen will contribute significantly to the reduction of leaching only when the fertilizer dose is better adjusted to the plant requirements.

The problems which have arisen as a result of the manure surpluses can be alleviated as follows:

- reduce the livestock through levy and subsidy policies;
- decrease the nitrogen in the fodder (see above);
- increase the sale of manure;
- use different methods of manure processing.

The reader is referred to the Manure Action Programme, as presented to the Lower Chamber on 24 February 1987, for a more detailed review of (a) the measures prepared and taken within the context of the manure problem, (b) the activities which are already under way or will be undertaken shortly, and (c) the consequences of a few matters for the livestock farmers and the environment.

7.1.3. Waste materials

Prevention

Sewage sludge

The N content of sewage sludge is determined by the composition of the treated sewage and the purification process used. It is difficult to alter the composition of the sludge. On the other hand, the application of different processes is in principle a possibility. Much research is being conducted into anaerobic processes, which produce less sludge. Clear prospects for this exist with certain concentrated waste-water flows.

Domestic waste materials

It is difficult to influence or reduce both the N content and the volume of waste products. Leaching of nitrate can however be limited by taking measures to isolate the waste in the case of storage, so that it is sealed from the surrounding environment.

Spoil from dredging operations

The N content of dredged spoil can be reduced by cleaning up waste-water discharges. The volume of dredged spoil cannot really be cut because this will directly affect the navigability of the waterways, but the effects on the environment can be limited through adequate storage and making provisions for isolation.

Recycling/beneficial application

When N-containing waste products are applied beneficially on or in the soil, the N present will generally be utilized by plants for cell growth. The nitrogen not taken up by the plants will, depending on the soil conditions, escape into the atmosphere as NH_3 or N_2 , leach into the groundwater or surface water, or undergo denitrification.

Composted domestic waste or sewage sludge, composted or not, can in principle be used as such. A problem with this, however, is the possible presence in the compost or sludge of substances harmful to the environment. It is also important that dosing is such that the nitrogen is taken up by the plants as far as possible. The extent and time of dosing (growing season) play an important role here.

Another problem is that by using sewage sludge, compost and the like as a fertilizer, the surplus of animal manure becomes even larger.

Processing/disposal

Processes such as (controlled) dumping and storage, composting and incineration are commonly used to process N-containing waste products. When waste is dumped and stored, natural microbiological processes take place in both the waste and the soil, such as fermentation, mineralization, nitrification and denitrification. Gaseous as well as soluble nitrogen compounds are produced which can be emitted. However, these can be limited by proper layout of the landfill site and treatment of the emissions that occur. Similar emissions arise with composting. These emissions can be reduced and controlled by appropriate process operation and layout. In incineration it is especially gaseous emissions such as NO_x which play a role. Their occurrence is determined by the type of waste to be burned and the combustion conditions to be applied (temperature, excess air). Important here are process selection and process operation. Only sewage sludge and domestic waste products are suitable for incineration. To date, sewage sludge is incinerated in only one plant in the Netherlands. It is likely that this will be done on a larger scale in the future. When dumping and storing waste, provisions for isolation can limit the emission into the surrounding environment.

7.2. SURFACE WATER

Nitrate and other nitrogen compounds end up in surface water as a result of

- industrial activities, 7 ktonnes per year;
- sewage treatment plants, 34 ktonnes per year;

- households, 9 ktonnes per year.

In addition, a nondescript amount of nitrate can indirectly reach the surface water through leaching and runoff. When dredged spoil is dumped back, nitrate can also end up in the surface water. The reduction of nitrogen emission from industry, through adaptation of processes and process operation and by emission-controlling activities, will not be discussed here in view of its small contribution to the total. The loading of surface water with nitrogen from waste-water discharges can be reduced by transporting the waste water to an rwzi where the nitrogen is then removed. There are several techniques for extracting nitrogen compounds from waste water (see table 7.1.).

However, most of these methods are not specific for nitrogen compounds. Of the techniques mentioned, some are never or seldom used, on grounds of cost. Techniques such as stripping and ion exchange are suitable only for some industrial waste-water discharges with relatively high nitrogen concentrations. In view of the relatively low nitrogen levels in domestic waste water, only the biological nitrification-denitrification method can be used in rwzi's, both on financial grounds and because of practical realizability.

Table 7.1. Removal or conversion of nitrogen compounds from waste water (STORA, 1985)

	Nitrogen compound	Overall selectivity
<u>Physicochemical methods</u>		
segregate contaminants by:		
- evaporation	NO_3^-	nonspecific
- expulsion (stripping using steam or air)	NH_3	specific
- reversed osmosis	$\text{NH}_4^+, \text{NO}_3^-, \text{urea}$	nonspecific
- adsorption (activated carbon)	org.N compounds	nonspecific
- ion exchange	$\text{NH}_4^+, \text{NO}_3^-$	partly specific
- electrodialysis	$\text{NH}_4^+, \text{NO}_3^-$	nonspecific
<u>Chemical methods</u>		
destroy contaminants by:		
- oxidation by combustion	Kjeldahl-N	nonspecific
- oxidation with chlorine	Kjeldahl-N	nonspecific
<u>Biochemical methods</u>		
convert contaminants by:		
- dissimilation, assimilation	all forms of nitrogen	N and P
- nitrification	NH_4^+	specific
- denitrification	NO_3^-	specific
- hydrolysis	org.N comp., urea	nonspecific

Biological nitrogen elimination

Nitrification in the activated sludge process is brought about by bacteria of the genera *Nitrosomonas* (conversion of ammonia to nitrite) and *Nitrobacter* (conversion of nitrite to nitrate). These bacteria have a low growth rate so that nitrification occurs only when the sludge is old, as in:

- lightly loaded activated sludge plants;
- lightly loaded oxidation beds;
- oxidation channels.

Bacteria capable of bringing about denitrification are prevalent in sludge. An oxygen-free zone in the sludge-waste water mixture is essential for denitrification to occur, because the bacteria then use nitrate instead of oxygen to oxidize organic matter. In this process, nitrate is reduced to nitrogen gas. Both nitrification and denitrification are essential for the complete biological elimination of nitrogen. The two processes require different process conditions. In addition, decomposition of organic compounds from waste water must also take place in biological sewage treatment plants. These three processes require in principle different microorganisms. Accordingly, various process configurations are possible for biological purification processes with nitrogen elimination. Their schematic diagrams are shown in figure 7.1. Of these systems, 2B, 3B, 3C and 3D are the most attractive from the point of view of cost and reliability.

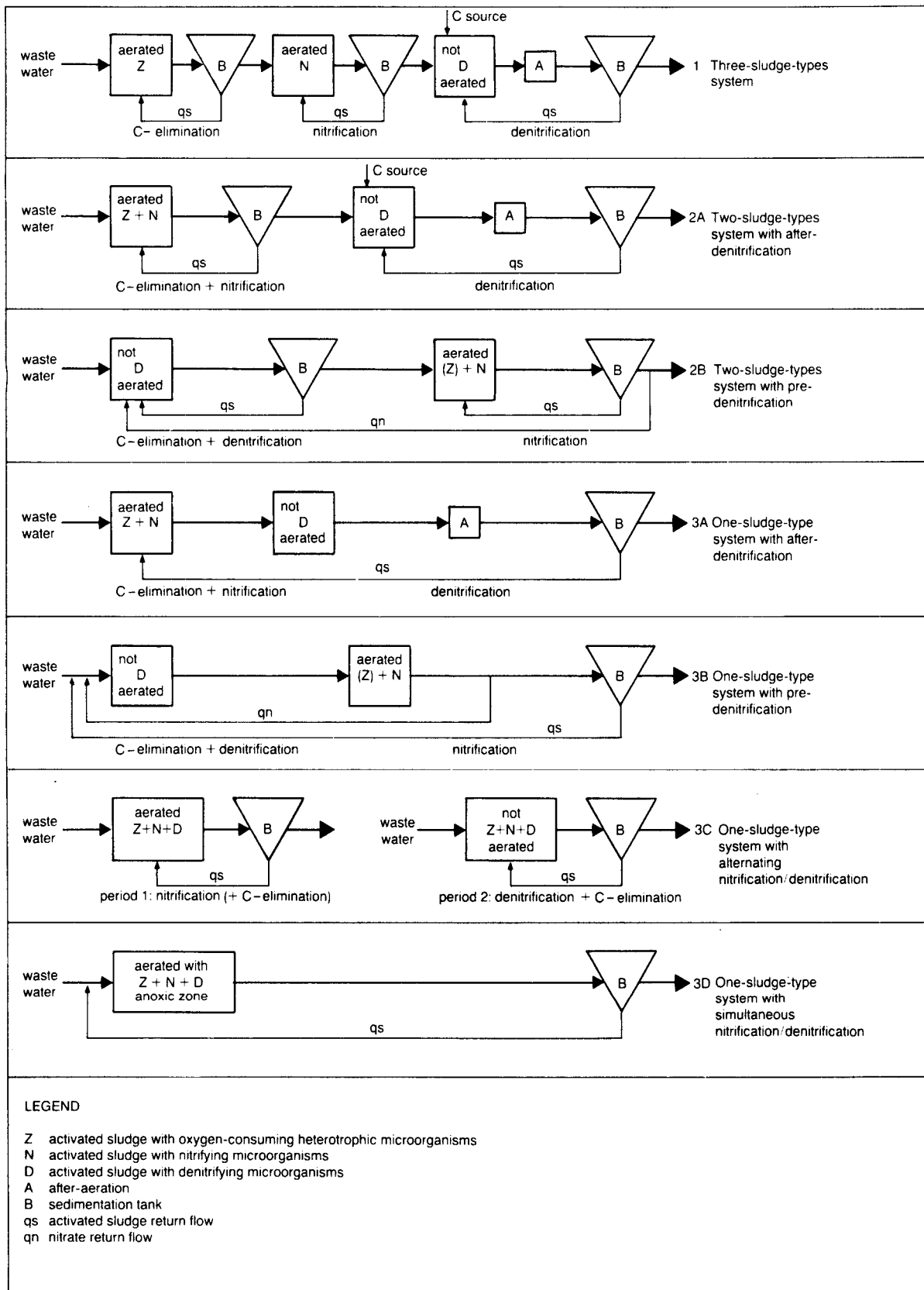


Figure 7.1. Suitable processes for biological nitrogen elimination (STORA, 1985)

Denitrification at the Dutch sewage treatment plants

In 1983, 460 sewage treatment plants were operational in the Netherlands. They have been categorized in table 7.2., stating whether or not nitrification and denitrification take place.

Table 7.2. Classification of biological sewage treatment plants

Type of rwzi	Number	Capa- city (%)	Nitrogen in surplus sludge	Supply (%)	
				nitri- fication	denitri- fication
Lightly loaded activated sludge plants	28	20	20	70	10
Heavily loaded activated sludge plants	37	25	20	10-20	0
Very lightly loaded activated sludge plants	280	30	10	80-85	60-80
Oxidation beds	87	14	20	15-25	0
Others	28	11	20	40-80	10-80

To reduce the emission of nitrogen compounds in the effluent of sewage treatment plants so that there is a total nitrogen elimination of at least 80%, measures must be taken which, depending on the type of plant, vary from process-integrated measures (aeration control) for lightly loaded activated sludge systems, to building on extra nitrification and denitrification areas in the case of heavily loaded activated sludge plants and oxidation beds. Table 7.3. summarizes the measures for improving total nitrogen elimination and the resulting additional cost (in guilders per i.e. per year). When interpreting the results it should be borne in mind that these are based on a particular design capacity and load level. The results therefore indicate only the order of magnitude of the anticipated extra cost.

Because of the requirements set for effluents, sewage treatment plants have generally been designed in recent years for extensive nitrification. In general, no requirements have been set for denitrification. A proportion of the investments mentioned in table 7.3. will in any case have to be made at a large number of these plants in the near future because of stricter discharge requirements.

Table 7.3. Measures for improving total nitrogen elimination and associated cost

<i>Type of rwzi</i>	<i>Measure</i>	<i>Extra cost in guilders per i.e. per yr</i>
<i>Very lightly loaded activated sludge plant</i>	<i>-aeration control</i>	<i>0 to 0.3</i>
	<i>-increasing recirculation</i>	
<i>Lightly loaded activated sludge plant</i>	<i>-building on denitrification tank</i>	<i>3 to 5</i>
<i>Heavily loaded activated sludge plants, oxidation beds</i>	<i>-building on nitrification and denitrification space and sedimentation tanks</i>	<i>10 to 15'</i>

7.3. AIR

The emissions of nitrogen compounds into the air have been described in chapter 2:

- production, storage and transfer of chemical fertilizer, 0.7 ktonnes per year;
- combustion processes, 154 ktonnes per year;
- livestock farming, 123 ktonnes per year.

7.3.1. Industry

Apart from combustion processes, the production, storage and transfer of commercial fertilizer are the only major industrial activities emitting nitrogen compounds. Of the 3810 tonnes of total dust, 3145 tonnes are released as fine dust during the prilling of chemical fertilizer. To control this nitrogen source, only the prilling of chemical fertilizer is being considered in view of its preponderant contribution. Prilling is a processing method whereby pellets are manufactured by atomizing a melt and allowing the molten droplets to solidify in the air while falling. It is employed in the manufacture of ammonium nitrate, calcium ammonium nitrate and urea. The dust emission is generally characterized by a very small mean particle size ($< 10 \mu\text{m}$, sometimes even $1 \mu\text{m}$), large volumes of cooling air (up to $1,000,000 \text{ m}^3$ per hour) and relatively low dust concentrations ($< 500 \text{ mg.m}^{-3}$). Theoretically, emission reduction is possible here by process

modifications such as fitting a "skirt" around the atomizer, which ensures that the fine dust produced during atomization is extracted and treated. A (large) part of the total dust can thus be extracted in a part-extraction. However, it is not clear to what extent the prilling process is adversely affected by such a process modification and how large the remaining emission will be. More practical research is needed. Emission control is receiving very little attention at present. Only in one instance is a spray tower in use for this purpose, with an efficiency of 90% (according to ERL) for total dust.

The following techniques are suitable: deep-bed filters with fibre packing, which are moistened continuously with water and cleaned by means of a washing cycle, high-pressure wet dust collectors (venturi washers), and cloth filters with special filter materials. A drawback of the first two methods is that they produce a waste-water stream. Although cloth filters are not yet used, it is believed that this technique is applicable in principle. The collection efficiency for total dust could be around 99%, with a residual concentration of $< 25 \text{ mg.m}^{-3}$. This means that the emission of fine dust will be reduced to 31 tonnes per year. When cloth filters are employed, provisions will have to be made in any case to enable the contaminated air flow to be passed through the cloth filters. This will entail considerable expense. To calculate the cost of emission control with cloth filters, the following "standard costs" were determined for the filter unit:

General	- standard cloth filter, glass-fibre cloth, applicable up to 120°C; - average cloth load, $80 \text{ m}^{-3}.\text{hour}$;
Investments	- including cloth material, ventilator, chimney, insulating material, cleansing system, ash removal capacity, + 50% installing cost - standard filter cloth: f 160.- per m^2; - special filter cloth: f 240.- per m^2;
Annual costs	- depreciation: 13.5 %; - maintenance: 2%; - insurances and the like: 1.5%; - energy consumption: $0.51 \text{ kWh per } 1000 \text{ m}^{-3} \text{ per hour}$; - energy cost: f 0.20 per kWh - operating time: 5700 hours per year; - filter replacement: after 2 to 2.5 years.

This means a capital outlay of 66.4 million guilders and annual costs of 24.3 million guilders when cloth filtration is employed in the prilling of chemical fertilizer. The cost per kg of trapped dust is therefore f 7.90 per annum for the filter unit alone. In addition to the filter system, several extra provisions are needed, such as ductwork and the installation of screens. It is expected that the cost of these provisions could be many times higher than that of the filter unit. Moreover, these costs can vary widely from situation to situation. The overall cost was therefore not estimated. It is likely that in the long term the prilling processes will be replaced by other, less dust-producing processes. This development has already begun.

7.3.2. Combustion processes

Stationary sources

The emission from combustion processes can in principle be controlled in two ways, one involving regulation of the combustion process so that less NO_x is formed and another involving removal of the NO_x formed from the flue gases by a so-called aftertreatment technique.

In small boiler systems with usually one burner and a relatively small furnace, the emission of NO_x can best be reduced by employing a low-NO_x burner and adjusting the boiler and burner accordingly. The application of low-NO_x burners can also cut the emission of NO_x in larger boiler and furnace systems. In addition, it is possible to suppress NO_x formation by changing the manner of firing, the so-called low-NO_x firing.

The following aspects are relevant to the special low-NO_x burners:

- low peak temperature, which reduces mainly the formation of thermal NO_x;
- shorter residence time, which cuts both thermal and fuel NO_x;
- no oxygen present where the temperature is highest;
- internal reduction, which also lowers thermal and fuel NO_x.

There is a diversity of measures to achieve this, such as split-flame burners, flue-gas recirculation, delayed mixing and so on.

Low-NO_x firing measures being taken in the operation of the plant (where required, after modifying the construction) have in part the same background as those for low-NO_x burners:

- a relatively low furnace temperature;
- the creation of reducing zones (e.g. by the so-called off-stoichiometric combustion process);

- as little excess air as possible.

In general, the measures required to achieve this in the furnace are far-reaching for existing plants and hence often too costly to implement. They are real options, however, for new plants.

The removal of NO_x from flue gases will not be practicable for anything other than large, and indeed only for very large, boilers because of the costs involved and the necessary technical provisions.

The following methods can be mentioned:

- selective non-catalytic reduction,

NO_x is reduced with NH₃ (H₂, CH₃ or CO can also be used) at a high temperature (about 1050 °C), reductions of up to 60% are feasible;

- selective catalytic reduction,

NO_x is reduced, usually with NH₃, with the aid of a catalyst, for which basic metal oxides based on TiO₂ or SiO₂ are used, the process cuts NO_x emission by 80-90%.

In addition, there are a number of other techniques, such as combined SO₂-NO_x removal, the oxidation-adsorption process and the absorption-reduction process, which are, however, of no practical importance at the moment.

In gas turbines, which also use burners for combustion, NO_x reduction is achieved by the same methods as described for low-NO_x burners. Delayed combustion and steam/water injection are the principal methods.

NO₂ emission from stationary engines can likewise be reduced by adjusting the combustion process in the cylinders or by employing a catalyst to clean the exhaust gases. The method of approach is in principle similar to that followed with the engines of mobile sources.

Mobile sources

Petrol engines

The emission from the combustion of petrol in engines can be cut sharply by using three-way catalytic converters. Another way to lower emissions is improved combustion in the engine, for example, by the application of the so-called lean-burn engine. The lean-burn engine is estimated to reduce NO_x emission by 50%, and by 70% when combined with exhaust gas recirculation. The catalytic afterburner cuts NO_x emission by 70%. The catalyst does, however, increase fuel consumption slightly.

LPG engines

The use of LPG as a fuel reduces NOx emissions significantly when the engine has been properly adjusted. It is expected that constructional modifications to petrol engines aimed at reducing emissions (lean-burn engines) will also achieve this objective when LPG is used. It may be assumed that the costs will be the same.

Diesel engines

Catalytic after-treatment of the exhaust gases is not possible because diesel engines operate with excess air. The most appropriate approach to the reduction of emissions is altering the method and timing of the fuel injection (e.g. pre-chamber injection). The combustion at the highest temperature then takes place under fuel-rich conditions, with negligible formation of NOx. However, this adversely affects fuel consumption, which increases by 1% to 2%. The emission of NOx can be cut by 50%. Because of the long useful life of commercial vehicles and ships, the effect of such measures will only become apparent in the long run (dozens of years) (Thomas et al., 1983).

Assuming that three-way catalysts will be incorporated into all passenger cars in the Netherlands, then the cost of this measure can be estimated as follows (based on 1983 figures):

- cost of a three-way catalyst per car (excluding trade margin): f 700.-;
- number of cars with a petrol engine in 1985: 5 million;
- total capital outlay: f 3,500 million.

Annual costs:

- depreciation (5 years, 6% interest rate): f 830 million per year;
- extra fuel consumption in relation to the autonomous development in fuel-efficient engines (at f 1.50 per litre): f 430 million per year.

The depreciation expenses are currently not passed on directly to the consumer owing to a change in the tax regimen and adjusted price-fixing by manufacturers and importers.

Total annual cost when three-way catalysts are applied: f 1,170 million per year.

For a more detailed description of possible measures to reduce NOx emission, their cost, and advantages and disadvantages, consult the "Note on the Acidification Policy interim evaluation" (1987).

7.3.3. Agricultural sector

The major emissions of NH_3 arise from:

- storage of manure and ventilation of the animal sheds (57 ktonnes NH_3 -N per year);
- spreading of manure on the land (39 ktonnes NH_3 -N per year);
- manure production during the grazing period (27 ktonnes NH_3 -N per year).

Storage of manure and ventilation of the stables

The current methods for limiting NH_3 emission are:

- improvement in the housing and manure-treatment systems;
- treatment of ventilation air.

The measures in the field of housing and manure treatment are aimed at preventing the transfer of ammonia from the manure to the air. A few examples of such measures are:

- half-slatted floors for pigs;
- partially slatted floors;
- storage of manure in an enclosed space, possibly in combination with daily manure removal from the stables;
- floor insulation of broiler barns;
- covering of silos;
- desiccation of laying-hen manure;
- manure belt batteries with forced drying instead of desiccation.

Biowashing and biofiltration are techniques used for treating ventilation air.

A standard agricultural biowasher consists of a packed column; the bacteria which break down the absorbed substances grow on the packing material. The efficiency of NH_3 removal is 80% to 90%. The cost varies between f 0.30 and f 0.40 per m^{-3} .

This amounts to f 12.- to f 17.- per pig delivered, f 1.50 to f 3.- per year for laying hens.

Several dozen biowashers are currently operational in the Netherlands.

In biofiltration, the contaminated air is passed through a bed containing biologically active material such as compost, which removes 75-95% of the ammonia from the ventilation air of pig and poultry houses.

The cost is estimated (rather optimistically, and based on installation by the farmer) at f 15.- to f 30.- per pig and f 12.- to f 16.- per 1000 eggs produced (TNO, 1985; DHV, 1986).

Manure transport and application to the land

The emission of NH_3 during manure spreading can be reduced by manure injection. The injection of pig slurry was found to cut emission by 90%. However, this also results in increased leaching of nitrate from the manured land. On the other hand, nitrate deposition on and leaching into adjacent nature reserves would then decrease again.

The extra cost is estimated at a few guilders per m^{-3} of manure. Emission reduction after the manure has been applied to the land can further be achieved by ploughing it into the soil quickly.

The extent of emission reduction depends on the type of manure, the weather conditions and the interval between application of the manure and ploughing it into the soil.

Manure production during the grazing period

At present, the emission of NH_3 can only be reduced by limiting the number of dairy cows. As a result of the 6.6% superlevy imposed in 1984, the stock of dairy cattle will decrease and the emission of NH_3 is expected to fall correspondingly (TNO). The May 1986 counts reveal that it has indeed fallen by some 7% since 1984.

Another possibility is adjusting the animal fodder. By letting the cows eat more maize instead of grass, the nitrogen excreted will decrease and thereby the ammonia emitted. A third possibility is to keep the cows indoors for a longer period. Although this does not reduce the emission, it has become at least manageable.

For a more detailed description of possible measures to reduce NH_3 emission, their cost, and advantages and disadvantages, the reader is referred to the "Note on the Acidification Policy interim evaluation" (1987).

7.4. DRINKING WATER

It will become clear in a number of cases that the nitrogen/nitrate emission cannot be limited sufficiently and/or fast enough to prevent too much nitrate from entering the drinking water ($> 50 \text{ mg NO}_3^- \cdot \text{l}^{-1} = 11.3 \text{ mg NO}_3^- \cdot \text{N} \cdot \text{l}^{-1}$). In these instances the nitrate concentration will have to be reduced during the production of drinking water. Suitable purification techniques which can at present be used in the preparation of drinking water are:

- ion exchange;
- hyperfiltration;
- biological denitrification.

Research is furthermore being conducted into the possibilities of denitrification in situ.

Of these techniques, a brief description will be given of the process involved, sensitivity of the process, aftertreatment and the waste problem.

7.4.1. Ion exchange

To remove nitrate, which is dissolved in the water as an anion, strongly basic anion exchangers can be employed. In the ion-exchange process Cl^- is usually exchanged for NO_3^- . In general, apart from NO_3^- , HCO_3^- and SO_4^{2-} will also be present, which compete with NO_3^- for binding to the ion exchanger. Which ions are preferentially bound depends on the ratio between the concentrations and on the selectivity of the ion exchanger. A conventional ion exchanger has a greater selectivity for sulphate than for nitrate.

In ion exchange, the treated water can have a very high chloride/hydrogen carbonate ratio, a high pH and corrosive properties. pH correction may be necessary to reduce the risk of corrosion. Another, but more expensive, solution is to regenerate the ion exchanger again with a carbonate solution following its regeneration with NaCl.

Other possible problems are bacteriological growth on the supporting material (resin) and the release of organic compounds from the resin or slow disintegration of the resin. Aftertreatment consisting of filtration over activated carbon or disinfection may then be necessary.

In ion exchange, the nitrate is liberated as a waste stream in the brine of the process. This brine with high nitrate, sodium chloride and, possibly, sulphate concentrations must be disposed of. Transport to the sea is costly and transport to an rwzi will encounter difficulties because of the high NaCl concentration in the brine.

A totally different approach is the use of another exchange principle and/or the use of other regeneration chemicals. An example of this is the application of an ion exchanger in the hydrogen carbonate form. Nitrate ions are here exchanged with hydrogen carbonate ions. Regeneration is done with calcium carbonate and carbon dioxide. This method offers advantages as regards both cost and disposal of the regeneration solution (Van Beek et al., 1984).

7.4.2. Hyperfiltration

Hyperfiltration produces water with a low salt concentration from brackish or salt water by forcing the water under high pressure through a membrane, the properties of which are such that the water can pass through but not the greater part of the ions.

In practice, membranes which remove about 90% of the nitrate are chosen, for reasons of cost. Hyperfiltration converts 75-90% of the water into product. The remaining water, the so-called brine, should not be further concentrated in order to limit contamination of the membranes. Prior removal of suspended and colloidal substances from the raw groundwater is necessary to prevent clogging and damage of the very expensive membranes.

Dissolved iron and manganese too, which can form precipitates on the membrane, should be removed beforehand. Precipitation of calcium phosphate can be prevented by lowering the pH. In addition to nitrate, other ions such as chloride and sulphate are also removed. The concentration of hydrogen carbonate also falls while that of CO_2 rises sharply. After hyperfiltration, the water is free of salt and aggressive as regards composition. The water can be given the following aftertreatment:

- mixing with water which has not been desalted, followed by pH correction;
- filtration over limestone.

The yield, i.e. the volume of reclaimed water as a percentage of the volume of raw groundwater, is fairly low (about 75-90%). This means that a considerable waste stream arises, amounting to 10-25% of the groundwater volume. All the salts intercepted by the membrane are concentrated in this so-called brine. Disposal of this brine and the associated cost are a major problem.

7.4.3. Biological denitrification

Nitrate can be converted to nitrogen (N_2) by bacteria under anaerobic conditions. The rate at which bacteria convert nitrate is limited. It is therefore necessary to create the right conditions so that the process becomes applicable in practice. This implies that the treatment plants must be provided with a large surface area to which the bacteria can attach. It is also essential to attain a satisfactory rate of material transfer.

For all types of design, the rate of nitrate removal depends on the particular substrate used (methanol is preferred), the temperature and the biomass concentration in the reactor.

The formation of CO_2 and N_2 gases in the reactors can be an interfering process. In fluidized-bed reactors, this will cause material to float so that more biomass ends up in the water produced. In solid-bed reactors, channels may develop in the bed so that short circuits are possible.

Problems with nitrite accumulation are encountered in autotrophic denitrification. Long residence times are required to remove all nitrate and nitrite.

In heterotrophic denitrification, all types of reactor run the risk of breakthrough of nitrate or methanol or of leaching of the biomass. In autotrophic denitrification, the water produced may contain nitrite and leached biomass.

Because of these factors and the fact that the water product is oxygen-free, extensive aftertreatment by aeration, activated carbon filtration and disinfection are necessary.

The biomass formed in the reactor must be partly removed from time to time by rinsing. The rinse water can be treated or discharged into the sewer.

7.4.4. Other techniques

In addition to the above-mentioned purification techniques, nitrate can also be removed by electrodialysis and flash evaporation. Both techniques are desalination processes having the same advantages and disadvantages as hyperfiltration.

Chemical reduction and denitrification with the aid of immobilized enzymes are processes which may be developed in the future to the stage at which their application becomes feasible.

Another possibility is the combination of two nitrate extraction techniques viz. ion exchange and biological denitrification.

The principle of this combined process is based on the elimination of nitrate from groundwater by means of an ion exchanger, whereafter the ion exchanger, when it is loaded with nitrate ions, is regenerated biologically with the aid of denitrifying bacteria. Treatment of groundwater (ion exchange) and regeneration of the exchanger (biological denitrification) are carried out separately. This system has been developed by the Agricultural University of Wageningen.

Another technique which is already being applied on a small scale at a few sites abroad is denitrification in situ, in which an organic carbon source

is injected into the soil. Sucrose, ethanol or methanol are generally used as the carbon source. The extent of denitrification observed varies between 50% and 90%. In some trials, e.g. in the Netherlands, problems with clogging were encountered (Mercado et al., 1987; Janda et al., 1987; Braester et al., 1987).

7.4.5. Implementation of denitrification systems at water companies

The costs of denitrification systems have been calculated from the following basic data:

- the purification capacity is 300 m³ per hour;
- the nitrate level of the groundwater is 100 mg.l⁻¹;
- the drinking water contains 25 mg nitrate.l⁻¹.

The cost for the various systems is: (costs including depreciation, chemicals, energy, maintenance; investments including pre- and aftertreatment and sludge processing/brine treatment):

- ion exchange: f 0.45 - f 0.75 per m³;
- hyperfiltration: f 1.- - f 2.- per m³;
- biological denitrification: f 0.40 - f 0.50 per m³;
- AU Wageningen system: f 0.55 - f 0.65 per m³.

The cost of ion exchange and hyperfiltration is heavily dependent on the brine treatment, with discharge into a sewer or surface water as the cheapest extreme and evaporation or discharge into the sea the most expensive.

Biological denitrification, and thus also the AU Wageningen system, produces biomass. The treatment cost can vary between f 0.05 per m⁻³ (discharge into a sewer) and f 0.15 per m⁻³ (sludge processing).

7.5. SUMMARY AND CONCLUSIONS

The possibilities of limiting the emission of nitrogen into the soil from the use of commercial fertilizer and animal manure are numerous, varied and sometimes complex. Only in the longer term can an improvement in the quality of soil and groundwater be expected from the implementation of various measures.

Examples are:

- better adjustment of the fertilizer dose to the plant requirements;
- cultivation of aftercrops;
- application of nitrification inhibitors;
- adaptation of the manure spreading regimen;
- change in fodder composition.

Leaching can be reduced by the provision of ground cover after the growing season, the use of nitrification inhibitors, adjustment of the manure spreading regimen and increasing the moisture content of the soil.

The use of nitrification inhibitors and changing the manure spreading regimen are worthwhile only after the manure dose has been adjusted to the plant requirements.

The emission of nitrogen into the soil from the dumping of wastes can only be reduced to a limited extent. The actual emission can be cut, however, by making provisions for isolation (application of IBC criteria). Nitrogen loading of surface water due to discharges from rwzi's can be cut by about 80% by adding a nitrification/denitrification stage to the rwzi's. At present, about 35% of the N supplied is removed. The cost can vary between 0 and 15 guilders per inhabitant equivalent per year. A relatively small amount of nitrogen is emitted from the prilling of chemical fertilizer. There is hardly any experience with control techniques and their introduction is hampered by the very high cost involved, whereas the emission is insignificant in comparison with that from other sources. NO_x emission from combustion processes can be limited by employing low-NO_x burners and applying aftertreatment techniques. A reduction of 60% to 90% can then be achieved. An NO_x reduction of 50 - 70% is considered feasible for mobile sources through modifications to engines. The emission of NH₃ by the agricultural sector can, as far as the emission from animal houses is concerned, be reduced by improved storage of manure, a different type of housing or by treatment of the ventilating air. A reduction of 95% can be achieved with the last-mentioned measure. The emission of NH₃ from manure spreading can be limited by injecting the manure or ploughing it into the soil quickly. However, these measures will increase the emission of

nitrogen into the soil and consequently the leaching of nitrate. Little can as yet be done about the emission of NH_3 during the grazing period.

In a number of cases the nitrate concentration in the drinking water will be or will become too high. Several purification techniques are applicable, viz. ion exchange, hyperfiltration and biological denitrification. All these techniques have major disadvantages. On the other hand, the combination of ion exchange and biological denitrification has important advantages. However, this combined process has not yet been developed far enough for application in practice. The cost of drinking water treatment varies between f 0.40 and f 2.00 per m^3 . An alternative possibility is denitrification in situ. Although trials in the Netherlands were hampered by signs of clogging, denitrification in situ is being successfully applied abroad, with nitrogen removal percentages between 50 and 95%.

8. FINANCIAL CONSEQUENCES OF EMISSION REDUCTION

8.1. INTRODUCTION

The production and application of nitrate and other nitrogen compounds were reviewed in chapter 2. The production within industry is concentrated in the fertilizer plants. Manure derived from intensive livestock farming is a major source within the agricultural sector. The extent of emissions from industry is limited. Agriculture produces the largest emission. There is a manure surplus especially in those regions where pig and poultry farming has developed, which gives rise to emissions upon storage and spreading.

Possible emission-controlling measures and their costs were discussed in chapter 7. Additional measures can be taken at the fertilizer plants at an annual cost of f 24 million.

More general measures to reduce nitrogen emissions are removal of nitrogen in sewage treatment plants and removal of NO_x from flue and exhaust gases. Purification of groundwater containing too much nitrate will also entail costs. Since these measures are not aimed at specific industrial sectors, and their costs will in part weigh directly on the consumer, statements about financial consequences at industrial-sector level cannot be made.

This chapter will consider the financial consequences of the proposed measures. Each industrial sector has to be discussed separately on account of their different natures as well as differences in environmental measures. First, an outline is given of the nature, size and composition of the fertilizer industry, poultry farming, pig farming and dairy farming (structural outline). The MIOU method ('t Gilde et al., 1986) has been used to gain an insight into the functioning of each sector. This method distinguishes three key variables: market situation, international competition and staying power. The value of the key variables is determined in relevant sections on the basis of a number of distinctive features of each industrial sector. The three variables, considered in connection with one another, characterize concisely the situation in which the particular industrial sector finds itself. This situation determines the leeway which companies have in reacting to externally imposed measures. Finally, this leeway is placed alongside the environmental cost estimated in the previous chapter. In this way an attempt is made, for each industrial sector, to

arrive at a prediction of how the entrepreneur will react to the proposed environmental measures.

The results for the four industrial sectors will be set alongside each other at the end of this chapter.

To conclude, a limitation attached to this chapter should be pointed out. It is quite possible that an industrial sector also emits substances other than nitrate, against which measures are also under consideration. This chapter does not provide insight into the possible accumulation of measures and their financial consequences.

8.2. FERTILIZER PLANTS

8.2.1. Structural outline

The fertilizer industry manufactures chemical fertilizers on a large scale for agriculture, which are used to increase the yield of crops and to prevent exhaustion of the soil. The fertilizers are distinguished according to the nature of the active ingredients into nitrogen, phosphorus and potassium fertilizers. In addition, there are mixed fertilizers consisting of a combination of the three constituents mentioned. The production of nitrogen-containing fertilizers accounts for 80% of the total volume, making it by far the most important category. Nitrate is present in 98% of the nitrogen and mixed fertilizers used in the Netherlands (Rinsema, 1985). The Netherlands has 8 fertilizer firms. Amfert (a subsidiary of the Israeli company ICL, based in Amsterdam) specializes in phosphorus-containing fertilizers. The other firms produce mainly nitrogen-containing fertilizers. They belong to five companies: DSM-Meststoffen (a subsidiary of DSM, with manufacturing units in Geleen, IJmuiden and Pernis), the Nederlandse Stikstof Maatschappij (NSM, a subsidiary of the Norwegian company Norsk Hydro, based in Sluiskil), Kemira (a subsidiary of the Finnish company Kemira Oy, based on the island of Rozenburg), Windmill Holland, based in Vlaardingen, and Zuid-Chemie (a subsidiary of the French company CdF, based in Sas van Gent). The fertilizer industry is a division of large chemical concerns and a considerable part of it is foreign-owned. In addition to DSM, Norsk Hydro, Kemira Oy and CdF, the production in Western Europe is controlled by ICI (Britain), BASF and Hoechst (West Germany) and Enichem (Italy).

The turnover of the Dutch fertilizer industry was f 3600 million in 1985, or 9% of the total chemicals industry. The five above-mentioned firms accounted for about 95% of this, i.e. f 3400 million. A total of 5400 persons were employed in the fertilizer industry in 1985. This is 600 fewer than in 1979. The average sales of over f 500 million per company as well as the complete absence of small firms indicate very clearly that it here concerns a very large-scale industrial sector.

8.2.2. Market situation

Chemical fertilizers are produced in bulk according to standard specifications. Since commercial fertilizers can furthermore be readily transported, we are dealing with a world market. For example, India and China are major markets for DSM-Meststoffen (DSM annual report, 1985). The strong international competition creates a high price sensitivity. Over the past 7 years, the market for nitrogen fertilizers has shown large price fluctuations: in 1979 the price was on average f 0.25 per kg. In 1981 the price rose to f 0.38 per kg, to fall again in 1983 to f 0.29 per kg. In 1984 and 1985 the price level of 1981 was reached again. Accordingly, this is reflected in an alternation of good years (1980 and 1981) and bad years (1982 and 1983). Since individual companies have a negligible influence on the market price and the large-scale, specialized manufacturing plants are not very flexible, riding on the international waves is inevitable.

The market for chemical fertilizers in the Netherlands and the EEC is no longer growing. Fertilizer use in the Third World, on the other hand, has grown rapidly since the 1960s. The increased demand was met by new factories in these countries themselves, so that exports from the Western countries remained at the same level and did not grow. Since a number of natural-gas producing countries (East bloc, Middle East) have expanded their production capacity for nitrogen fertilizers, there is now an overcapacity for these products worldwide. The East-bloc countries compete heavily on the West-European market in order to earn "hard" currency. At present, the oil-producing countries in the Middle East are trying to gain a firm foothold on the Third-World markets. For the Dutch companies, therefore, the market seems to be saturated (CPB, 1986).

Dutch companies have for years supplied more than 90% of the domestic market. This market share fell between 1979 and 1984 to around 75%. This trend changed direction in 1985 (DSM, 1985). Competitiveness in the

West-European scene is good to reasonable, thanks to the central location in the market region and the Dutch natural gas reserves. The market share within the EEC has been consolidated over the past few years, but the Dutch fertilizer industry is losing its competitive edge in the markets outside Europe. The trend over the past few years as a whole has been one of a slight fall in market shares.

The price sensitivity, market situation and falling market share discussed above all point in the direction of a weak market situation.

8.2.3. International competition

The last section already mentioned that the market for chemical fertilizers is a very international one. The Netherlands is one of the largest exporters in the world. In 1985, exports accounted for 64% of the output, with a value of f 2300 million. Of this, 18% went to the Third World, for the most part in the form of urea. Urea is a non-nitrate-containing nitrogen fertilizer. The remaining exports are aimed especially at the EEC countries West Germany, France and Britain.

The value of the imports, which in 1979 was 17% of the export value, rose to 27% of the exports in 1983 (f 489 million). Imports amounted to f 531 million in 1985, or 23% of the export value. Imports come mostly from EEC countries, especially Belgium, West Germany, France and Spain. Although the export surplus has decreased relatively over the past 7 years, in absolute terms it still involves a very large amount of money.

The above figures show that international competition is very important for the fertilizer manufacturers.

8.2.4. Staying power

The profitability of the fertilizer industry is very unstable. This is related to the large price fluctuations on the world market. The good years 1974 and 1975 were followed by three bad years, while 1979 to 1981 inclusive were good, but 1982 and 1983 bad again. The upward trend in 1984 did not continue in 1985 (DSM, 1985). The operating results were about 10% of the invested capital in the good year 1981, but were zero in the following years.

They had recovered to about 5% in 1985, which should be deemed low.

Solvency can only be considered in a meaningful way at concern level. With 24% of the balance sheet total being private capital, DSM is not very solvent.

The variable costs of the fertilizer plants amount to 70% of the production cost. The major item is natural gas, which is used in two ways: as an energy source (14%) on the one hand, and as a feedstock for the manufacture of ammonia (20%) on the other. The price of natural gas doubled between 1979 and 1981 in the aftermath of the second oil crisis. It was possible to offset this so long as world fertilizer prices were still increasing in 1980 and 1981, but as a result of the fall in prices in 1982 the companies immediately made a loss. The slump in energy prices in early 1986 obviously pushed down the variable costs considerably. The value added comprises about 25% of the production value. Labour costs account for two-thirds of this. The manufacturing plants of Dutch industry are very modern and efficient. The depreciation of these large-scale, complex and largely automated plants accounts for 5% of the production cost. The fertilizer industry is all in all a capital-intensive, very energy-intensive and not a very labour-intensive sector.

The average low profitability, fairly weak solvency and high sensitivity to fluctuations in market and natural gas prices distinguish the fertilizer industry as an industrial sector with weak staying power.

8.2.5. Summary and conclusions

The fertilizer industry is a large-scale industrial sector. Both the market situation and staying power are weak, while international competition plays a very large part. It is therefore not surprising that, in view of the uncertain and virtually static environment, companies can only survive within large concerns.

A rise in costs of f 24 million per year resulting from environmental measures amounts to nearly 1% of the sales income, or 3.5% of the value added. This represents a fairly large additional financial burden. Because of the inflexibility of the plants this will not have major consequences for the production in the short term. In view of the low labour intensiveness it is also not expected that employment will be directly affected. In the long run, however, replacement investments and thereby employment may be jeopardized.

8.3. POULTRY FARMING

8.3.1. Structural outline

Two main sectors can be distinguished in the poultry industry: farms keeping laying hens for the production of eggs and farms keeping broiler chickens for the production of meat. A third, smaller and supporting sector are the farm enterprises which keep dams for the production of broilers.

Lastly, there are also duck and turkey farms. They are not discussed here because they comprise less than 2% of the poultry industry.

The number of chicken farms is steadily declining; there were still about 50,000 in 1970 but this number had fallen dramatically by 1980, to 8681. The keeping of a small number of chickens as a sideline all but ceased during these years. The rate of decrease has since slowed down and the number of farms in 1985 was 7151, including 4534 laying-hen farms, 1477 broiler farms and 624 farms keeping dams for the production of broilers.

Poultry farming is the chief means of support for about two-thirds of the enterprises (1553 laying-hen farms and 493 broiler farms). In 1975 there were still 1777 enterprises with laying hens and 1075 businesses raising broilers as their principal activity.

Unlike the number of farm enterprises, the number of birds has increased over the past 10 years. The number of laying hens grew by 50% from 29 to 44 million. The number of broiler chickens remained constant and fluctuated around 39 million. The picture emerging from the above figures is one of a process of scaling-up and specialization. There was a relatively smaller decrease in the number of enterprises in the laying-hen than in the broiler sector, which is probably connected with the vigorous growth in the number of laying hens.

Poultry produce had a value of f 2713 million in 1985. Of this, eggs accounted for f 1348 million, broilers for the remaining f 1365 million. Total poultry sales accounted for 12% of the entire livestock industry, or 8% of the total agricultural turnover (f 34,000 million in 1985).

The value of the output of the poultry industry did not increase between 1981 and 1985. The importance of egg production has relatively diminished. The ratio of the value of eggs compared to that of meat was 5:4 in 1980, and in 1985 the scales had tipped slightly in favour of meat production.

Of the poultry farms, 96% are run as family businesses, that is, only the owner and his family work on the farm. Such a business does not have hired workers. The family farm is thus predominant. The non-family enterprises (so-called mammoth enterprises) are on average 3 to 4 times larger than the family businesses. In 1983, the former category accounted for 15% of the laying-hen and 8% of the chicken population.

8.3.2. Market situation

Because of the structure of the market (many relatively small suppliers) the individual entrepreneur has no influence on the market price. For him, the price elasticity at a particular moment is infinitely large. This means that he will not get rid of his products if he tries to sell above the market price but also that there is no point in selling below the market price because he will then be disadvantaged by too low proceeds. A consequence of the structure outlined is that the entrepreneur can only influence his income by adjusting the amount produced. He will do this by increasing or decreasing the number of birds.

The market price for poultry products has over the past few years lagged behind the average price for agricultural products. The price index in 1984 (1980=100) for eggs was 104 and for broilers 111, whereas this figure had risen to 118 for the agricultural sector as a whole. The prices for broilers kept up in 1985 but the egg prices fell by 10%. To maintain income, a strong impulse developed in the laying-hen sector to expand the number of birds. This was possible by investing in large-scale and labour-saving batteries. Fully 95% of the laying hens are now kept in battery cages.

The market share of Dutch producers on the domestic market is over 80% for both eggs and meat. There are no indications that this strong market position is under pressure.

The markets in the Netherlands and the EEC are saturated. A major cause of this is that population growth has all but ceased. In addition, the income elasticity for foodstuffs is generally less than one so that, as income increases, the relative proportion spent on food lags behind (CPB, 1986). Eighty-five per cent of the poultry production is sold on the European market. Expansion is in fact only possible by trying to open up new markets outside Europe. This will be discussed in more detail in the section on international competition.

The market situation for the poultry sector as a whole is weak: competitiveness is good, but both the volume and the market prices are stagnating.

8.3.3. International competition

The poultry sector is a major exporter within the Dutch agricultural industry. In 1983, 73% of the production, with a value of f 1996 million, was exported. Eggs and meat had an equal share in this.

The Netherlands is the largest exporter of eggs in Europe. The EEC countries, especially West Germany and Belgium, were and still are by far the most important customers. Over the past 15 years, the poultry sector has also been successful in building up a market for eggs and day-old chicks in North Africa and the Near East. Since these countries (especially Algeria and Iran) have to cut their imports as a result of falling oil income, a collapse of this market is envisaged (Het Parool, 1986). The prospects of new markets outside the EEC are few because it concerns here fragile products with high transport charges.

The import of eggs and poultry meat is less than 8% of the export. Over two-thirds of this comes from the neighbouring countries Belgium and West Germany. On balance there is thus a large export surplus amounting to over f 1800 million per year.

International competition is very important. This is attributable to the exports as imports are low.

8.3.4. Staying power

As regards the following, the fact that the poultry sector consists for the most part of family businesses should be kept in mind. As has already been mentioned, the development of the market price is a constant for the poultryman. On the basis of the development of this price he will try to produce as cheaply and, within the structure of his family enterprise, as much as possible.

The profits in the poultry sector are low. Sales and prices stagnate. Since the prices of the principal item of outlay - feed - increased by 20% between 1980 and 1984, the proceeds are barely sufficient to offset the costs, let alone provide a reasonable family income. Whereas livestock farming as a whole realized a gross operating profit of 19% in the business

year 1982/'83, it was 0% for the poultry sector (CBS, 1985a). This means that in the latter case the enterprises used up their capital. As a result of a 9% drop in feed prices in 1985, the situation for the broiler farmer in particular improved, but his income is by no means yet reasonable.

Considered within the framework of livestock farming, the poultry sector is not very solvent. Whereas private capital commonly amounts to 50% of total capital for the livestock industry as a whole, it is on average only 30% in the poultry sector. The other two major sources of financing are long-term loans (with a large role played by the Rabo banks) and short-term credits (25%). The latter percentage is exceptionally high within agriculture and indicates a heavy dependence on creditors.

The nature of poultry farming as a "food-upgrading industry" is clearly reflected in the large item of outlay for the purchase of feed (80%). Energy accounts for 3% of the production cost. This percentage is higher for the broiler sector because the animals are kept in an artificial environment. Because of the nature of the family enterprises - which do not hire workers - labour costs are negligible (2%). The scaling-up, which involved substantial investments in buildings, has left its marks in the depreciation expenses, which account for 5% of the production costs. Poultry farming can be characterized as a moderately capital- and energy-intensive industrial sector. It is not very labour-intensive, even when allowing for an entrepreneurial remuneration of about 10% (see section 8.3.4.).

The overall verdict on the staying power of this sector is that it is weak. Its negative profitability, its not very strong solvency as well as its susceptibility to the development of feed prices point in this direction. The structure of this sector (family businesses) can explain the pressure for continuity and even for expansion of production.

8.3.5. Summary and conclusions

Poultry farming emerges from the above as an industrial sector confronted with a labile market situation and strong international competition (chiefly attributable to exports). Its staying power is weak. Owing to the dominant position of the family business, there is a pressure for continuity.

8.4. PIG FARMING

8.4.1. Structural outline

The pig industry concentrates on the production of pork. On the stock farms, the breeding sows farrow and the weaners grow up here until they are 16 weeks old. After 16 weeks, the pigs are transferred to the the fattening farms where they are finished. The distinction between breeding and fattening establishments is not absolute. A combination of the two activities is common within one enterprise (so-called closed farms).

Table 8.1. summarizes the number of enterprises in the pig industry for certain years.

Table 8.1. Number of enterprises engaged in pig farming, classified according to type (CBS/LEI, 1986).

Type	1980	1984	1985
Stock farm	6,353	5,481	5,755 (4,806 chief occupation)
Fattening farm	4,496	4,193	4,474 (2,310 ")
Closed farm	1,474	1,423	1,514 (1,351 ")
Total	12,323	11,097	11,743 (8,467 chief occupation)

A striking feature is that the decrease in the number of enterprises, in itself a normal phenomenon in agriculture, changed to an increase in 1985. Pig farming is the chief means of support for over two-thirds of the enterprises. For stock farms and closed farms, the proportion of chief-occupation enterprises is significantly higher than for fattening farms. Stock farming is a specialized sector.

The number of animals has grown continuously over the past 10 years. Growth accelerated between 1984 and 1986. Table 8.2. summarizes this development.

Table 8.2. Number of pigs (x mln) (CBS/LEI, 1986; 1986a)

	1980	1984	1985	1986
Piglets < 20 kg	3.5	3.9	4.4	4.8
Pigs 20 - 50 kg	2.5	2.8	3.1	3.2
Porkers > 50 kg	2.9	3.1	3.4	3.8
Breeding sows	1.2	1.3	1.4	1.6
Total	10.1	11.1	12.4	13.5

A weight of 50 kg roughly corresponds to an age of 16 weeks. There were thus 8 million piglets in the stock farms in 1986 compared with 6.7 million in 1984 (+ 20%).

Table 8.3. shows the development in the value of the pig industry's output. The picture is likewise one of steady growth.

Table 8.3. Value of the pig industry's output (CBS/LEI, 1986)

<i>Year</i>	<i>Value of output (x mln fl)</i>	<i>Slaughters (x mln)</i>
1980	4,732	13.2
1983	5,837	14.8
1984	6,072	15.5
1985	6,685	-

The family business predominates in the pig industry. Only the owner and his family work in such enterprises and no workers are hired. This sector can therefore be described as being small-scale.

8.4.2. Market situation

Because of the structure of the market, with many relatively small suppliers, the individual entrepreneur has no influence on the market price. For him, the price elasticity at a particular moment is infinitely large. Since the price is a constant, the pig farmer can only influence his income by adjusting the amount produced, that is, by increasing or decreasing his pig population.

Pig prices rose by 17% between 1980 and 1984. This percentage is in line with the general price development in agriculture. Since the pig industry's profitability developed favourably as a result of lagging increases in cost, many pig farmers decided to invest in new sheds.

The Reduction of Pig and Poultry Farms Interim Act, which aimed to halt the expansion of intensive livestock farming, and thereby the manure surpluses, came too late to prevent this. The new pig houses were put into use in 1985, so that the number of animals rose by over 20% (see table 8.2). Under the influence of the increased supply of fatstock, the prices stabilized in 1985 (LEI, 1986). The oversupply of pork in 1986 led to a 15% drop in prices. A significant improvement in prices is unlikely in the coming years.

of the domestic market has been over 90% for years. There are no trends indicating that this strong market position is under pressure. Between 1973 and 1982 the Dutch share in the value of the EEC's production of pork rose from 10.6% to 15.2% (LEI, 1986). Productivity rose sharply due to intensification of the production. As a result, profitable production remained possible at prices which lagged behind those abroad, and this made it possible to increase foreign-market shares. The markets in the Netherlands and the EEC are saturated. A major cause is the almost zero population growth. In addition, the income elasticity of the demand for pork is less than one (CBS, 1986). The price elasticity of the demand for pork lies between 0 and -1. With the unfavourable market prices of 1986 and 1987, this can give some compensation through an increase in the demand. The market situation for pig farmers was reasonable to good up till 1985. Since 1985, this sector has reached the limits of the market although its competitiveness has not weakened.

8.4.3. International competition

In 1985, the pig industry exported f 4,887 million, or 73% of its output. Pork accounted for f 3,743 million of the exports and live animals (3 million) for f 1,144 million. The 15% increase in the number of live pigs exported may indicate that pig fatteners are gradually switching to breeding (Schothorst, 1986). Ninety-eight per cent of the exports go to other EEC countries, especially France, Belgium, West Germany, Italy and Spain (CBS, 1985a).

The volume of the imports is only a fraction of that of the exports, and had a value of f 178 million in 1984. The major suppliers were Britain and Belgium. On balance there was thus an export surplus of f 4,700 million. In view of the large export share, international competition is very important for the pig industry.

8.4.4. Staying power

Table 8.4. gives an idea of the development of the earnings in the pig industry. The earnings per breeding sow (average livestock in a chief-occupation business, 110 breeding sows) gives a picture of the breeding sector and that per porker (average livestock, 100 porkers) of the fattening sector.

Table 8.4. Earnings per animal per year (in fl) (LEI, 1986).

<i>Year</i>	<i>Breeding sow</i>	<i>Porker</i>
1980/81	50	11
1981/82	430	62
1982/83	640	69
1983/84	245	14
1984/85	515	71
1985/86 (estimate)	520	80

The results thus fluctuate wildly, in the fattening sector even more than in the breeding sector. The entrepreneur can realize a reasonable income in good years, but in bad years the enterprises have to use up their capital. Despite the low sales income, the results did not plummet in 1986 because the prices of the largest item of outlay, livestock feed, fell by 17% between 1984 and 1986.

The solvency of the pig farms is low. In 1984, private capital was on average only 9% of the total capital (NMB, 1986). External capital plays a dominant part in the financing of this sector. The pig industry requires much capital. So much money is invested in the livestock that the turnover rate of the capital is less than one. The character of pig farming as a "food-upgrading industry" is reflected in the large item of outlay for livestock feed (70%). The intensification and scaling-up of production have left their marks in the depreciation expenses, which account for 5% of the production costs. Because of the nature of the family enterprise, wage work is uncommon in the pig industry. Consequently, labour costs as an expense category are negligible (1%). When, to get a better picture, the remuneration allocated to the entrepreneur is added to this, then the labour costs are 11%.

Pig farming emerges from the above as a moderately capital-intensive industrial sector and not very labour-intensive.

The pig industry invested f 990 million during the investment boom of 1984/85. To date, the environmental costs in this sector have been low. The Manures Act, which came into force on 1 January 1987, will make it necessary to spend large sums on manure storage and manure transport in the years ahead.

The staying power can be characterized as weak.

8.4.5. Summary and conclusions

The pig industry comprises 7,000 family businesses. The market situation, which was reasonable to good for many years, deteriorated after 1985. International competition is strong and the staying power is weak.

8.5. CATTLE FARMING

8.5.1. Structural outline

The main line of activity in the livestock industry is dairy farming. The principal dairy products are milk, cheese, butter, skimmed milk powder, evaporated milk and whole milk powder. In addition, the production of veal (white meat) and beef (red meat) play a role.

It is not possible to make a distinction between a milk- and a meat-producing sector. Dairy farms also supply meat. On the other hand, calf-rearing can be distinguished as a separate sector. This is because it is part of intensive livestock farming, milk is not produced, and veal and beef have different markets (Neuteboom, 1986).

Table 8.5. gives the number of enterprises in the cattle industry for certain years (CBS, 1986a). The number of farms continues to decrease.

Table 8.5. Number of enterprises in the cattle industry, classified by type

Type of enterprise	1980	1984	1985
Overall cattle industry	62,561	59,032	56,030
Cattle farming as chief occupation	55,639	52,607	50,218
Dairy farms	57,471	54,836	51,710
Calf-rearing farms	1,573	1,503	1,482

The number of farms keeping cattle decreased further in 1986. On the other hand, the number of enterprises keeping feeder calves (calf-rearing farms and nonspecialized farms) rose from 2676 in 1985 to 2847 in 1986 (CBS, 1986a). The number of farms keeping cattle as a sideline has fallen during the past 10 years, partly because of the large investments which had to be made when pail-milking was discontinued (LEI, 1986).

A process of concentration took place within the whole cattle industry. Nevertheless, there are still many small farms in the dairy sector (Van der Giessen, 1986). In 1984, 71% of the feeder calves were kept on calf-rearing farms. This percentage had risen to 77% by 1985. In 1984, 88% of the bulls were kept on dairy farms (CBS, 1985b; 1986b).

The number of cattle increased up till 1985. As a result of the introduction of the superlevy in April 1984, the number of dairy cows and heifers as well as the total number of cattle fell in 1985 and 1986. Table 8.6. shows this development (CBS, 1980; 1985b; 1986a).

Table 8.6. *Composition of the cattle population (x 1000 head) (CBS, 1980; 1985b; 1986a)*

Type of cattle	1980	1984	1985	1986
Dairy cows and heifers	2356	2549	2367	2288
Young cattle	1908	1933	1845	1699
Feeder calves	582	638	638	689
Total	5226	5516	5248	5123

The value of the cattle industry's production fell in the year 1984/85. The sales revenue from milk was expected to increase in 1985/86 but that from beef to decrease further (see table 8.7; LEI, 1982; 1986).

Table 8.7. *Value of the cattle industry's production (including internal deliveries) (x mln guilders)*

	1980/81	1983/4	1984/85	1985/86*
Cattle farming	10130	13000	12145	12250
- milk	7710	9890	9320	9550
- meat	2420	3110	2825	2700
calf-rearing	1090	1810	1760	1810

* estimate

Because of the decrease in the cattle population, in response to the superlevy, meat production is under pressure. Another effect is a decreasing supply of newborn calves to the calf-rearing farms.

8.5.2. Market situation

There are a large number of relatively small suppliers in the cattle industry. The market structure is determined to a large extent by EEC regulations. Beef and milk are considered to be the heavy market-ordering products (LEI, 1986). The regulations applicable to the cattle sector include a common base price for milk with a fat content of 3.7% (f 0.73 per kg), intervention prices for butter and skimmed milk powder, a joint-responsibility levy (f 1.50 per 100 kg dd. 1-5/86) and threshold prices for

a large number of dairy products. To reduce the stocks of butter and skimmed milk powder, the superlevy was introduced in early April 1984. A farmer has to hand over 75% of the base price for milk as a fine for each kg of milk produced in excess of the quota allocated to him. In the Netherlands, the 1983 production level minus 8.6% was fixed as the farmer's quota for 1984/85. The quota was reduced by a further 1% in 1985/86. Up till 1989, the quota will be reduced by 3% (Van der Giessen, 1986; Krijger, 1986). Lastly, there are import levies for beef and beef cattle from countries outside the EEC (20 and 16% respectively) and intervention prices for beef cattle (CBS, 1986b; LEI, 1986).

Apart from large stocks of butter and skimmed milk powder, there are also large beef "mountains". The European Commission wants to abolish the automatic intervention for beef within two-and-a-half years (LEI, 1986).

Table 8.8. summarizes the price and income elasticities of meat and dairy products for the EEC-9 (Boddez and Emens, 1981).

Table 8.8. Price and income elasticities of beef, veal and dairy products for the EEC-9, for the period 1960-1979 and the year 1979 respectively

Product	Price elasticity	Income elasticity
Fresh milk		-0.05
Cream		1.02
Whole milk powder		0.24
Cheese		0.79
Beef and veal	-0.51	0.55

In 1985, the consumption of beef was, as in previous years, 27% and that of veal 3.5% (3.7% in 1984) of the total Dutch meat consumption.

Table 8.9 gives the market share of the Dutch farmers on the domestic market (LEI, 1986; Commodity Board for Livestock and Meat, 1984; 1985).

Table 8.9. Market share of Dutch farmers according to product and year (% of consumption)(1)

	1980	1983	1984
Dairy products(2)	50	41	43
Beef(3)	57	74	79
Veal(3)	88	94	94

(1) calculated as: $1 - (\text{import/export}) \times 100\%$

(2) in units of value

(3) in units of weight

The market share for dairy produce decreased slightly but that for meat increased.

The milk price rose until 1983 and remained practically constant thereafter. The prices for beef and veal rose in 1985 but not enough to offset the fall in the preceding year. The decrease in the prices in 1984 was caused by the sharp increase in meat production resulting from a large number of cattle having been slaughtered (superlevy!). Meat production fell again in 1985. Table 8.10. shows the price development (LEI, 1986).

Table 8.10. Price development of milk, beef and veal (% increase over the preceding year)

Product	1981	1983	1984	1985*
Milk	8.3	2.5	0.7	0.2
Beef	-	-2.6	-6.7	4.5
Veal	-	0.0	-6.1	3.0

* estimate

Given the large Dutch and EEC stocks of beef, butter and skimmed milk powder, the prospects of expansion of the total market are bleak. The sale of these stocks presented problems, so that the butter stocks increased despite a lower milk production (LEI, 1986). The per capita consumption of beef rose slightly and of veal fell slightly in the Netherlands and the EEC in 1985 (beef, 1984: 17.1 kg; 1985: 17.2 kg; veal, 1984: 2.4 kg; 1985: 2.2 kg).

Per capita domestic consumption of dairy products in 1984 was the same as in 1980. The consumption of skimmed milk powder in 1984 was less than in 1985. Butter consumption also fell in 1985 (1984: 3.9 kg; 1985: 3.6 kg). The export of milk and dairy products did not increase in 1985 but that of cheese and evaporated milk did (LEI/CBS, 1986; LEI, 1986).

The likely development of the dairy output is currently greatly determined by the EEC quotas and superlevy. Abolition of the automatic intervention is expected for the beef sector. For this reason, the market can be said to be weak.

8.5.3. International competition

The export value of dairy produce was f 7462 million in 1985, which means that it has stagnated since 1983. In 1985, cheese accounted for f 2495 million, butter for f 1468 million, evaporated milk for f 1301 million and

whole milk powder for f 958 million of the dairy export. Exports to other EEC countries had a value of f 4142 million in 1985, i.e. 56% of the total export. Major markets are Belgium-Luxemburg, West Germany, France (cheese), and Saudi Arabia (milk) (Commodity Board for Dairy Products, 1984).

The value of beef exports was f 1000 million (1980: f 500 mln; 1984: f 894 mln). In units of volume, the EEC accounted for 65% of the export, with France, Greece and the Federal Republic of Germany being the largest customers. Outside the EEC, Egypt is an important market (CBS, 1986a; Commodity Board for Livestock and Meat, 1985). Veal exports in 1985 had a value of f 1368 million (1980: f 896 mln; 1984: f 1187 mln). Virtually the entire export went to the EEC (over 90%), with Italy (50%) and the Federal Republic of Germany being the largest customers. In 1984, about 40% of the beef and about 95% of the veal produced were exported (Commodity Board for Livestock and Meat, 1985; LEI, 1986).

Dairy imports had a value of f 2990 million in 1985, with skimmed milk powder (feed for calves) accounting for f 1047 million and butter for f 715 million. Almost 100% of the imports came from other EEC countries (LEI, 1986). Major exporters are Belgium-Luxemburg, West Germany, Ireland (butter) and France (cheese) (Commodity Board for Dairy Products, 1984).

Beef imports, chiefly from other EEC countries (76%), had a value of f 378 million in 1985, with Belgium-Luxemburg having the largest stake, 45%.

Veal imports are of minor importance: f 24 million in 1985, chiefly from Belgium-Luxemburg.

Table 8.11. gives the ratio between import and export for the various products.

The international market is very important for the cattle industry.

Table 8.11. Ratio between import and export (%) per product

<i>Product</i>	<i>1980</i>	<i>1983</i>	<i>1984</i>	<i>1985(3)</i>
<i>Milk(1)</i>	<i>38</i>	<i>49</i>	<i>48</i>	<i>40</i>
<i>Beef(2)</i>	<i>112</i>	<i>57</i>	<i>39</i>	<i>38</i>
<i>Veal(2)</i>	<i>2</i>	<i>1</i>	<i>1</i>	<i>2</i>

(1) in units of value (LEI, 1982; 1985a, 1986).

(2) in 1000 kg of meat; Commodity Board for Livestock and Meat

(3) from the figures given in the text

8.5.4. Staying power

The profitability of the dairy farms is given in table 8.12. as the ratio between the earnings of the entrepreneur and the revenues. It shows that profitability remained low in 1984/85. The spread is large: 20% of the larger and 34% of the smaller businesses had negative earnings in 1985 (LEI, 1986). An improvement in profitability is expected for the year 1985/86 resulting from slightly higher prices and costs remaining constant (LEI, 1986).

Table 8.12. Profitability of dairy farms (x 1000 guilders)

Period	<u>Large enterprises</u>		<u>Small enterprises</u>	
	earnings of the entrepreneur/ revenues	% of revenues (1)	earnings of the entrepreneur/ revenues	% of revenues
1980/81	17.6-25.0(2)	6- 8	7.7	6
1983/84	20.6-37.8	6-11	5.9	4
1984/85	27.4-42.0	8-12	7.6	5
1985/86(3)	34.0-44.0	-	12.0	-

(1) revenues calculated from the ratio of costs to revenues to the net surplus

(2) the range indicates differences in average profitability for different regions and soil types

(3) estimate

Profitability in the calf-rearing sector, given in table 8.13., is too low for running a cost-effective enterprise and is, moreover, still decreasing. This is due to a larger increase in the prices of newborn calves than of fattened calves.

Table 8.13. Profitability of calf-rearing farms (guilders per calf)

	80/81	82/83	83/84	84/85	85/86
Earnings of entrepreneur(1)	71	80	73	57	50
Net profit (2)	1164	1400	1414		
Earnings/net profit	6%	6%	5%		

(1) LEI (1986)

(2) LEI (1985b)

The solvency of grassland farms in 1984 was between 60% and 82%, depending on size, type of ownership and location (see table 8.14.; LEI, 1986; CBS, 1986). Since dairy farming makes up the largest part of this category, these figures can also be used for the dairy farms. No data are available for the calf-rearing farms.

Table 8.14. Solvency of various types of grassland farms in 1984
(private capital/total capital x 100%)

Type of enterprise	Solvency
Ownership, large	65.4-80.5*
Ownership, small	82
Leasehold, large	60.7-72.5
Leasehold, small	81

* depending on the location of the farm

Feed consumption accounted for nearly 50% of the total costs incurred by dairy farms in 1983/4. Feed prices show a downward trend (LEI, 1986). Table 8.15. gives a few items of expenditure of dairy farms (CBS, 1986b).

Table 8.15. Items of expenditure in dairy farming (x mln guilders)

	1982/83	1983/84
Wages of hired personnel	132	149
Family members	366	422
Work done by third parties (including pesticides, herbicides, etc.)	105	98
Depreciations	928	1036
Livestock feed	4045	4973
Total	9338	10529

The ratio between depreciations and labour costs (including estimated earnings of the entrepreneur) was 60% in 1983/84. Cattle farming is a capital-intensive sector. No data could be found for the calf-rearing sector.

The overall verdict on the staying power of the dairy farms is that it is weak. Solvency is strong but profitability is low. Although a complete picture of the calf-rearing farms cannot be given, their low profitability points to weak staying power.

8.5.5. Summary and conclusions

Cattle farming is an industrial sector confronted with a weak market situation. Import and export play an important role. The staying power, with the exception of solvency, is weak.

8.6. DISCUSSION AND CONCLUSIONS

The most important data on the four industrial sectors discussed above have been conveniently arranged in table 8.16.

For each sector, the increase in costs resulting from the measures proposed in chapter 7 are also indicated, as far as they are known.

Table 8.16. Overview of industrial sectors

<i>Sector</i>	<i>Structure (size of enterprise)</i>	<i>Market situa- tion</i>	<i>Inter- national competition</i>	<i>Staying power</i>	<i>Increase in cost (% of value added)</i>
<i>Chemical fertilizer</i>	<i>large</i>	<i>weak</i>	<i>strong</i>	<i>weak</i>	<i>3.5%</i>
<i>Poultry farming</i>	<i>small</i>	<i>labile</i>	<i>strong</i>	<i>weak</i>	
<i>Pig farming</i>	<i>small</i>	<i>labile</i>	<i>strong</i>	<i>weak</i>	
<i>Cattle farming</i>	<i>small</i>	<i>weak</i>	<i>strong</i>	<i>weak</i>	

Given the values of the key variables, all four sectors have an unfavourable starting position. They differ substantially in scale: large (fertilizer) versus small (poultry, pig and cattle farming).

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ADDENDUM INDUSTRY

Report A 88.070, 1988.02.15

Addendum to the Integrated Criteria Document
"Nitrate" by the RIVM

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HUMAN TOXICITY

The Integrated Criteria Document "Nitrate" as a scientific basis for the effect-directed standardization policy raises many questions.

For example, chapter 5 gives an overview of the literature on the effects of nitrate, nitrite and nitrosamines on the health of man. However, careful study reveals that only minimal attention has been paid to recent and highly relevant literature despite the fact that earlier remarks referred to this. As a result, the following problem factors were barely discussed or even overlooked in the risk analysis:

1. the endogenous formation of nitrate in both humans and animal studies;
2. methaemoglobinaemia in children was not critically evaluated in connection with point 1;
3. the results of epidemiological studies were not discussed adequately and these studies were not rated at their true value. Points for and against were all that were mentioned.

re 1. The study of Hegesh and Shiloah (1982) showed that in young children with intestinal infections the endogenous synthesis of nitrate can increase to 15 mg per kg body weight. Comparing this figure with the proposal in the Integrated criteria document "Nitrate" for the lowest oral dose of 1.5-2.7 mg nitrate per kg body weight, there is here an enormous discrepancy, especially when it is considered that this lowest oral dose was mainly derived from studies in patients suffering from gastrointestinal infections in whom the contribution from endogenously synthesized nitrate can be high.

re 2. The Integrated criteria document "Nitrate" described methaemoglobinaemia as a phenomenon caused by the process: nitrate intake-nitrate reduction-oxidation of haemoglobin. The data on methaemoglobinaemia cases concern almost without exception infants with

intestinal infections and diarrhoea. However, the Integrated criteria document only established a relationship with the nitrate concentration in the drinking water. This interpretation is a denial of the scientific fact that the cases of methaemoglobinaemia described in the literature should be regarded as a multifactorial disorder, whereby the following factors play a role (see also appendix 1):

- gastrointestinal infection in the child;
- the nitrate-reducing capacity of the bacteria that cause the gastrointestinal infection;
- bacterial contamination of drinking water and food which can bring about nitrate reduction and gastrointestinal disturbances;
- the amount of endogenously synthesized nitrate in the body and, in addition, the amount of nitrate taken in via the food.

When discussing methaemoglobinaemia, it is of vital importance to distinguish between chemical contamination by nitrate, and bacteriological contamination. Therefore, the study by Cornblath and Hartman (1948), who gave healthy infants without gastrointestinal infection "clean" water containing high concentrations of nitrate (50 or 100 mg nitrate per kg body weight), should definitely also be discussed.

It is remarkable that the Integrated criteria document "Nitrate" paid so much attention to the extrapolation of animal studies, putting forward as an argument that "no suitable data are available on the semichronic or chronic exposure of man to nitrate or nitrite on which a no-toxic effect level can be based". This type of data can be found, for example, in the study of Bruijns (1982) which was not mentioned in the integrated criteria document. In this study, 268 kidney patients received daily ammonium nitrate equivalent to 100 mg nitrate per kg body weight during a period of from three weeks to two years. No methaemoglobinaemia was observed, except in two patients: it was due to a deficiency of methaemoglobin reductase in one patient, while treatment was discontinued in the other.

The pure nitrate effect emerged from both the study of Cornblatt and Hartman (1948) and that of Bruijns (1982), and for a clear understanding of methaemoglobinaemia it is essential to discuss and evaluate this type of study in relation to the other studies.

re 3. The discussion of the results of the epidemiological studies is inadequate. A 1985 WHO report paid much attention to this type of studies from the past. The WHO concluded that there was no conclusive evidence of a

link between gastric cancer and consumption of drinking water containing more than 45 mg of nitrate per litre.

The WHO stated that most epidemiological studies were not adequate because the nitrate-"treated" group was either not described properly or too small. To overcome this objection, the British government conducted a large-scale study, comparing a group of 1400 workers at a nitrate factory with the "normal" population (Al-Dabbagh et al., 1986). This recent research has not been discussed adequately in the Integrated criteria document "Nitrate" whereas the conclusions are clear: no difference in risk of stomach cancer was established between the group of 1400 workers and the control group from the population.

In summary, the human toxicological effects were not discussed adequately in the Integrated criteria document "Nitrate": publications were either not discussed at all or not related to the derivation of the lowest oral toxic dose of nitrate. Important studies were unjustifiably not mentioned. This is in our opinion unacceptable for the Integrated criteria document "Nitrate", which serves as a scientific basis for the effect-directed standardization policy of the Dutch government.

ECOTOXICITY

In the treatment of the subject "Ecotoxicity" in chapter 5, a so-called safety factor (Kooijman) and an uncertainty factor were used. These factors may be used only when they are based on differences in sensitivity among species. However, this is by no means the case in surface water, because differences in test medium, test duration, toxicity mechanism and metabolism (degradation, bioaccumulation) all play a part. It is therefore scientifically unacceptable to use this method for determining a "safe" value for surface water.

FINANCIAL CONSEQUENCES

The discussion of the financial consequences of emission reduction concerned a few industrial sectors only. This is inappropriate for a correct assessment of the priorities.

The disproportionate attention given to the fertilizer industry with the advice to cut back its current emission of about 0.6 ktN per year is unwarranted when the total emission into the air is 290 ktN a year in the Netherlands. As is apparent from the accompanying letter to the Integrated

criteria document "Nitrate", this huge imbalance and the disproportionate increase in costs have also been noticed by the authors. Accordingly, the fertilizer industry was not included in the proposal for taking environmental measures in view of its small contribution to the total emission (!).

It would be appropriate if this imbalance were also rectified in the Integrated criteria document.

Literature:

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See also appendixes I and II

SPECIFIC COMMENT

p VII:

- The fertilizer industry received disproportionate attention in view of its small contribution of less than 0.5% to the total air emission.
- Suggestion for the 1st sentence, last paragraph:

As regards the financial consequences for relevant agricultural activities, such as cattle farming, etc.

p 25: In our opinion table 2.14. should be as follows:

<u>Source</u>	<u>Soil</u>	<u>Water</u>	<u>Air</u>	<u>Total</u>
Fertilizer industry		4.5	0.7	5
Other industries		2	25	27
Central heating			34	34
Traffic			98	98
Livestock farming	270		123	393
Chemical fertilizer application	111		8	119
Households	1	9		10
Sewage treatment plants	13	34		47
Waste products	65			65
Dredged spoil	60	840		900
Import	n.a.	549	72	
Export	n.a.	596	278	

p.137:

- Text, 14th line from the bottom should read:

There are 8 fertilizer firms in the Netherlands: Amfert (a subsidiary, etc.) and Windmill Holland (a subsidiary of the Norwegian company Norsk Hydro, based in Vlaardingen) both specialize in phosphorus-containing fertilizers. The other firms produce mostly nitrogen-containing fertilizers.

They belong to four companies: DSM-Meststoffen (a subsidiary etc.), the Nederlandse Stikstof Maatschappij (NSM, also a subsidiary of Norsk Hydro, based in Sluiskil), Kemira (a subsidiary etc.) and Zuid Chemie (a subsidiary etc.).

- 2nd line from the bottom: Omit Hoechst, as it has virtually ceased fertilizer production.

p.138:

- first line: Omit "the five above-mentioned etc."

p 140:

- Text, 6th line from the bottom should read:

This is a large additional financial burden, the more so as it concerns only a cut in emissions of about 0.7 ktN/year out of a total emission into the air of 290 ktN/year.

In the long term, replacement investments can etc.

APPENDIX I

COMMENT ON APPENDIX TO REPORT NR 758473012: INTEGRATED CRITERIA DOCUMENT
NITRATE, EFFECTS

W.F. ten Berge

1. INTRODUCTION

The addendum gives an extensive review of the scientific literature on nitrates, nitrites and nitroso compounds.

Finally, a risk evaluation is made based on the studies reviewed.

However, the risk evaluation is strongly biased by

- lack of important studies on endogenous nitrate synthesis in humans and experimental animals during bacterial infections;
- lack of critical evaluations of methaemoglobinaemia cases in infants;
- lack of parameters other than nitrates, which strongly influence the cancer risk.

These shortcomings hamper an unbiased risk assessment of nitrates. The lacking information is presented below.

2. ENDOGENOUS SYNTHESIS OF NITRATE DURING BACTERIAL INFECTIONS OR INFECTIOUS CONDITIONS

2.1. Animals

Wagner et al. (1983) fed 6 rats 154 mg ¹⁵N-ammonium acetate and measured ¹⁵N-nitrate in the urine 24 hours after dosing. After a one-week interval, the same dose of ¹⁵N-ammonium acetate and, in addition, 1 mg E. coli lipopolysaccharides (LPS) per kg body weight were administered intraperitoneally to the same 6 rats. On average, urinary nitrate increased by a factor of 9 following LPS treatment, from 1.6 mg per kg body weight per day to 14 mg per kg body weight per day. LPS is an endotoxin which induces fever. Two other types of inflammatory states produced changes in nitrate biosynthesis. Intraperitoneal injection of 150 mg Carrageenan increased urinary nitrate excretion by 200%. Subcutaneous administration of 0.5 ml turpentine caused a delayed increase in urinary nitrate excretion of 300% after 3 days.

Stuehr and Marletta (1985) studied E. coli lipopolysaccharide-stimulated nitrate synthesis in LPS-sensitive C3H/He and LPS-resistant C3H/HeJ mice. Intraperitoneal injection of 15 μg of LPS led to a temporary 5- to 6-fold increase in blood nitrate concentrations in the LPS-sensitive mouse strain, but not in the LPS-resistant strain, even at an i.p. dose of 175 μg LPS. Injection of Mycobacterium bovis into mice from both strains resulted in a large increase in nitrate production over the course of the infection and increased urinary nitrate excretion from 3.6 mg per kg body weight per day to 164 mg per kg body weight per day on the 9th day following injection (average body weight of mice was assumed to be 34 g).

2.2. Humans

That nitrate can be excreted in excess of intake as a result of intestinal infections was recorded by Mayerhofer (1913) who noted that nitrate could not be detected in the urine of healthy breastfed infants, but even minor intestinal upsets quite often gave rise to nitrate-positive urine samples. Subsequently, Catel and Thunger (1933) found that infants fed only human milk excreted nitrate in detectable quantities. Nitrate excretion was attributed to endogenous synthesis; daily excretion varied greatly in the 10 infants studied. At that time, only semi-quantitative measurements could be made but the authors did note increased nitrate excretion by one infant suffering from enteritis.

Wagner and Tannenbaum (1982) observed an increase in urinary nitrate excretion from 43 mg to 280 mg NO_3^- per day in a human subject on a low constant intake of dietary nitrate of 7.4 mg/day after he developed nonspecific intestinal diarrhoea. The authors concluded that activation of the reticuloendothelial system significantly increased nitrate biosynthesis and suggested that it might be dependent on oxygen-radical formation.

Similar evidence has been supplied by Hegesh and Shiloah (1982). They determined blood nitrate, nitrite and methaemoglobin levels and urinary nitrate excretion in 58 hospitalized infants with acute diarrhoea. 130 Subjects of different age groups without gastrointestinal disturbances, including 30 infants, acted as controls. Although the control and diseased infants had been given the same food preparations with low nitrate content (2-7 mg NO_3^- per infant per day), blood nitrate levels were 7 times and urinary nitrate excretion 15 times higher in the diseased than in the control infants.

We evaluated their data and estimated the urinary nitrate excretion to be 1 mg NO_3^- per kg body weight per day for the control infants and 15 mg NO_3^- per kg body weight per day for the infants with diarrhoea.

Twelve of the diseased infants had a methaemoglobin level of more than 8% of total pigment, accompanied by a mean blood nitrate concentration of 37 mg NO_3^- per litre. The authors summarized their clinical experience with methaemoglobinaemia as follows:

"Infantile methemoglobinemia is of much more frequent occurrence among hospitalized newborns and young infants than is generally appreciated. When present, acute diarrhoea of different etiologies is almost exclusively the cause of hospitalization. No correlation between this condition and ingestion of food or water containing high concentrations of nitrates or nitrites was found. This study demonstrates for the first time that high blood nitrates are a regular part of the syndrome. The evidence presented is interpreted as indicating that diarrhoea results in endogenous, de novo synthesis of nitrites, and this is the principal cause of infantile methemoglobinemia."

Wettig et al. (1987) described a 25-year-old pregnant patient with lamblasis and a pathogenic *E. coli* infection with diarrhoea. Her nitrate intake was less than 30 mg per day; she had nitrate concentrations in morning saliva and urine of 700-900 mg per litre over 6 days and nitrite concentrations in saliva of 50-60 mg per litre during 8 days of observations. When the lamblia were eliminated by treatment with metronidazol, the nitrate concentrations fell to a normal level of less than 100 mg per litre. Other lamblasis patients also had elevated nitrate concentrations in urine, though not so extreme (149 ± 40 mg/l before treatment, 54 ± 40 mg/l after treatment).

2.3. Evaluation

The available evidence shows clearly that, in animals under inflammatory conditions, endogenous nitrate synthesis varies from 14 to 160 mg per kg body weight per day.

In humans, endogenous nitrate synthesis can easily reach a level of 15 mg/kg body wt/day, especially in infants. This is important for an objective judgment of the causes of infant methaemoglobinaemia.

The studies mentioned above should be inserted on page 7 and page 14 of the appendix of report nr. 758473012 Integrated Criteria Document Nitrate, Effects.

3. INFANTILE METHAEMOGLOBINAEMIA

3.1. Nitrate level of drinking water

Sattelmacher (1962) reviewed the 467 published cases for which water nitrate concentrations were available. His review includes the data from an earlier review by Walton (1951). Sattelmacher reported:

Nitrate concentration in water used for feed preparation ($\text{mg NO}_3^-/\text{l}$)

	0 - 40	41 - 80	81 - 100	> 100
Number of cases	14	16	19	418

These findings form the factual basis for the original water quality criterion that water with more than $100 \text{ mg NO}_3^-/\text{l}$ should not be used as potable water. This point of view is currently beyond dispute.

The enigmatic nature of the condition has been well expressed by Donahoe (1949):

"It is difficult to explain why only an occasional infant develops cyanosis, why the nitrate content of the water (associated with the cases) varies so greatly and why it is not always the water with the highest nitrate concentration which causes cyanosis in the infant."

3.2. Intestinal infections

Ceck et al. (1964), Worm (1964) and Zanisco (1964) appear to be the first to clearly describe cases of infantile methaemoglobinaemia as a complication of gastroenteritis in infants with low or negligible nitrate intake. In Worm's case the water used contained $1 \text{ mg NO}_3^-/\text{l}$, and in two out of Zanisco's 7 cases the infants were breastfed prematures (human milk contains less than 5 mg NO_3^- per litre).

In the study by Ceck et al. (1964), the infant excreted NO_3^- in its urine although oral nitrate intake had been excluded. Many other cases have

subsequently been described. The common features were diarrhoea (usually with green stools), metabolic acidosis, and cyanosis. No exposure to methaemoglobin-forming agents could be demonstrated, while nitrate intake was found to be low or absent.

In some cases the condition developed whilst the infant was hospitalized, cf: Caille and Herouin (1969), 3 cases; Barna et al. (1984), 9 cases; Yano et al. (1982), 11 cases; Bricker et al. (1983), 15 cases; Hanukouglu et al. (1983), 9 cases; Seeler (1983), 5 cases; Danish (1983), 5 cases; Blanc et al. (1983), 4 cases; Maloney et al. (1983), 1 case; May (1985), 1 case; Mailfert et al. (1986), 1 case; Lanir et al. (1986), 11 cases. Both Shearer et al. (1972) and Shuval and Gruener (1972) observed cases of elevated but subclinical methaemoglobinaemia associated with diarrhoea, but gave no details. Kohne et al. (1972) described two similar cases in whom enteritis was due to cow's milk intolerance.

The combination of methaemoglobinaemia and infant gastroenteritis can also occur as epidemic episodes. Fandre et al. (1962) treated 54 cases of infantile gastroenteritis in Reims (France), 19 of whom developed methaemoglobinaemia at different stages. Six cases with methaemoglobin levels varying from 15 to 59% of total haemoglobin were carefully studied. The infants were fed powdered baby feed suspended in tap water with apparently low nitrate content. The authors concluded that the methaemoglobinaemia originated from endogenous nitrite formation due to intestinal infection by enteropathogenic coliform bacteria.

Although well documented, enterogenous methaemoglobinaemia seems to be fairly rare. Berg and Rappe (1976), without giving details, stated that in Sweden 21 infants hospitalized with diarrhoea had methaemoglobin levels below 2%. Since Barna et al. (1974) found that only a few of the enteritis patients developed elevated methaemoglobin levels, a negative result serves only to indicate that methaemoglobinaemia is not the usual outcome of gastrointestinal upsets.

The origin and causes of this form of methaemoglobinaemia have only been sparingly researched. Hegesh and Shiloah (1982) showed that endogenously synthesized nitrate can cause infantile methaemoglobinaemia. They suggested that the condition could be the result of acute diarrhoea, due to enhanced nitrate synthesis caused by the enteritis, irrespective of nitrate intake.

Of recent interest is a gastrointestinal epidemic affecting 50 infants in Spain, resulting in cyanosis and methaemoglobinaemia (Farre Sostres et al., 1982). The community water contained 76 mg NO_3^- /l. The epidemic nature of this episode is similar to that reported by Fandre et al. (1962), where enteropathogenic *E. coli* were believed to be the causative agent of the methaemoglobinaemia. The Spanish clinical findings concerning gastroenteritis in combination with acidosis are similar to those described by Yano et al. (1982) and others, where the methaemoglobinaemia was a complication of gastrointestinal infection and not associated with nitrate intake.

It therefore seems likely that the nitrate content of the community water was not the main causative factor of the Spanish epidemic.

The occurrence of methaemoglobinaemia as a complication of infantile gastroenteritis raises the possibility that cases ascribed to nitrate in the water used to make the feeds may actually have been the result of infection.

This explains Sattelmacher's findings (1962) that, below a nitrate concentration of 100 mg/l of drinking water, there was hardly any increase in the incidence of methaemoglobinaemia with increasing nitrate concentrations.

3.3. Bacterial contamination of water

In 1945, Comley was the first to describe two cases of well-water methaemoglobinaemia in children 33 and 27 days old. The association between nitrate in water and the disease stems from this paper. Poor water quality was linked by circumstantial evidence to cyanosis and the failure of infants to thrive. He noted that the two infants were suffering from gastrointestinal infections and had been given feeds made with polluted water containing 388 and 619 mg NO_3^- /l respectively, and 1.3 and 0.4 mg NO_2^- /l respectively. There was heavy bacterial contamination, with 240 coliforms/100 cc.

The water had been drawn from shallow wells.

Comly described the situation as follows:

"The high nitrate water which the cyanotic infants ingested came from very undesirable wells. In many cases the wells were old, dug rather than drilled, had inadequate casings or none at all and were poorly covered so that surface water, animal excreta and other objectionable material could

enter freely. In every one of the instances in which cyanosis developed in infants the wells were situated near barn-yards and pit privies. Some of the wells had trees growing nearby, and the roots had penetrated or broken down the casings."

Nearly all cases of infantile methaemoglobinaemia were associated with the use of well water, mostly shallow well waters. Shallow wells are more easily contaminated by leaching of fertilizers and manure.

The nitrate level in well water was used as a measure of the extent of pollution. However, bacterial counts were hardly ever made in connection with infantile methaemoglobinaemia.

Nevertheless, the association between infantile methaemoglobinaemia and the use of shallow well waters suggests that both nitrate and bacterial contamination can contribute to methaemoglobinaemia in infants.

3.4. Evaluation

Infantile methaemoglobinaemia is a multifactorial disease. The following contributing factors can be distinguished:

- Intestinal infections in the infant.
- Nitrate-reducing capacity of the bacteria that cause gastroenteritis.
- Bacterial contamination of water and food, which may cause nitrate reduction and intestinal infections.
- Nitrate level of drinking water and food in addition to endogenously formed nitrate.

In the risk-analysis section on pages 103 and 104 concerning infantile methaemoglobinaemia, only exogenous nitrate intake and its reduction have been considered. However, the exclusion of exogenous nitrate intake by infants will not prevent methaemoglobinaemia though it may reduce its severity. The main cause appears to be bacterial infection within the intestines.

It has further been assumed on page 103 that 80% of the nitrate is reduced in infants. This is not consistent with page 13, where 80% to 100% of ingested nitrate is said to be excreted. Nitrate reduction of as much as 80% is very exceptional and will only occur when there is severe bacterial contamination of food or, in the case of severe intestinal infections,

result from nitrate-to-nitrite-reducing bacteria. These cases are rare. A lethal dose of 43.2 mg nitrate for babies is given on page 45. Such a dose can readily originate from endogenous nitrate synthesis (Hegesh and Shiloah, 1982).

4. CANCER RISK FROM EXPOSURE TO NITRATE

Only a few lines of text are devoted to the epidemiological studies relating nitrate intake and cancer incidence. The importance of these studies is appreciated more highly by the WHO (1985), who summarized the available evidence as follows:

"No convincing evidence of a relationship between gastric cancer and consumption of drinking-water containing nitrate levels up to 45 mg NO₃ per liter 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 inadequacy of the data available. Gastric cancer is declining in most countries, and the risk of nitrate, if any, would appear restricted to individuals with conditions 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 incidences at other sites."

The risk estimation on pages 105 and 106 is based solely on nitrate intake and its conversion to nitrosodimethylamine and nitrosodiethylamine.

The RIVM used the results of Oshima and Bartsch (1981) for estimating the conversion rate of nitrate to nitrosamines.

Oshima and Bartsch (1981) gave a volunteer 65, 130, 195, 260 and 325 mg NO₃ in beetroot juice together with 500 mg proline. The formation of NPRO (= nitrosoproline) in vivo was found to be strongly dependent on nitrate intake; when less than 195 mg nitrate were ingested, a marginal or in most cases no increase in urinary NPRO was observed, as compared with that in a control experiment. The formation of NPRO in a human from nitrate and proline in vivo increased exponentially with the dose of nitrate ingested, and linearly with the dose of proline when different amounts of proline were taken after ingestion of 325 mg nitrate. It is important to point out

that a single high dose of nitrate had no effect on urinary nitrosoproline excretion. This finding is consistent with observations in ferrets who received drinking water containing 500 ppm ¹⁵N-labelled nitrate. Only 2% of the labelled nitrate was incorporated into urinary NPRO. This indicates that there are at least two pathways, one of which not being influenced by dietary nitrate intake (Dull et al., 1986).

The conversion rate of nitrate to NPRO was 0.002% when 325 mg nitrate and 500 mg proline were ingested. This extent of conversion, which does not occur at normal dietary nitrate levels with and without additional proline, was adopted by the RIVM as the regular conversion rate of nitrate to NMDA (nitrosodimethylamine) and NDEA (nitrosodiethylamine) from reaction with dimethylamine and diethylamine respectively.

This reasoning is incorrect because

- at normal dietary nitrate levels, its conversion to NPRO, and likewise to NMDA and NDEA, does not occur to a measurable degree;
- conversion to NMDA and NDEA is 20 times slower than to NPRO (Mirvisch, 1975);
- the amount of secondary amines taken in via the diet is much smaller (< 20 mg) than the experimentally administered dose of 500 mg proline.

From an epidemiological point of view, the risk estimation is exaggerative. Vegetarians have the highest nitrate intake (270 mg versus 110 mg normally), because they consume large quantities of fresh vegetables (NAS, 1981). Vitamin C, present in the fresh vegetables, blocks nitrosamine synthesis (Oshima and Bartsch, 1981).

Vegetarians (Seventh Day Adventists) generally have a much lower cancer mortality than other people (Hirayama, 1986). Nevertheless, the RIVM's risk estimation would predict a higher cancer death rate for vegetarians, because of their relatively high nitrate intake, than for other people and is thus principally wrong.

5. RISK ASSESSMENT FOR ECOSYSTEMS (PAGE 107-110)

It is impossible to comment upon the actual experimental results presented, but the researchers could be questioned about the methods used regarding reference cultures and death rates of fish eggs and larvae.

The differentiation between salts of nitrate and the influence of other ions on toxicity raise more questions than provide answers in relation to natural waters, total ionic composition and salinity.

The determination of the safe concentration of nitrate in freshwater is based on the experimentally determined mean or lowest LC50, divided by a so-called safety factor (Kooijman, 1986) or uncertainty factor (Slooff et al., 1986).

The safety factor of Kooijman (1986) is derived from the extent of scatter of LC50 data for different species. The uncertainty factor of Slooff et al. (1986) is based on the residual standard deviation of the regression of the acute LC50 or EC50 at (to?) the highest no-effect concentration in chronic tests for a large number of substances and species of organism, reported in the scientific literature.

The use of the safety factor and the uncertainty factor would be meaningful if the scatter and standard deviation respectively were determined solely by differences in sensitivity among species.

However, the scatter and standard deviation are strongly influenced by:

- differences in toxicity mechanism
- differences in metabolism
- differences in test medium and the accompanying stress on the test organism
- differences in test duration, expressed in the generation period of the test organism.

Therefore, the use of the safety factor or uncertainty factor to estimate safe concentrations for the most sensitive species leads to extremely low limits which have no ecotoxicological but only statistical significance.

The proposed "safe" value for fresh digotrophic water of 0.04 mg NO_3^-/l (page 108) corresponds to about 5% of the mean value of oligotrophic lakes in Europe (see table below: most of the epilimnetic inorganic N consists of nitrate). Such proposals have to be characterized as meaningless.

Comments on the eutrophication considerations:

Denitrification is not a strictly anaerobic process but occurs when the oxygen concentration is less than 1-2 mg/l. Denitrification therefore takes place mainly in the water bottom, i.e. at the sediment-water interface and in the sediment. The process can even occur in water layers in which oxygen is plentiful. This phenomenon is attributable to surface films on organic particles in the water column.

Daily rates of denitrification of up to 200-300 mg $\text{NO}_3\text{-N/m}^2$ have been measured in lakes. The rates are correlated with the concentrations of nitrate.

In general, it can be pointed out that:

- Nitrogen concentrations in waters are to some extent self-regulating, and quantification and understanding of the processes taking part are of great importance for assessing the effects that can be obtained from reductions in input of nitrate.
- The nitrate-oxygen functions as an important electron acceptor in the mineralization process of organic matter, leading to a reduction of the oxygen deficit in the water masses. (The addition of $\text{Ca}(\text{NO}_3)_2$ to sediments exploits this effect through stopping the internal cycle of phosphorus in lakes, thereby reversing eutrophication.)
- Nitrogen fixation may be a significant process where $\text{N/P} < 7$. Such conditions favour the nitrogen-fixing blue-green algae (some species may be toxin producers), and blooms of them will become more frequent.

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APPENDIX II

THE DERIVATION OF ECOLOGICAL LIMITS FROM ECOTOXICOLOGICAL STUDIES AND THE APPLICATION OF UNCERTAINTY OR SAFETY FACTORS

W.F. ten Berge

INTRODUCTION

In the integrated criteria documents of the RIVM which, among other things, present a detailed description of the toxic properties of a chemical substance, one section always concerns ecotoxicological studies. For a number of species of water organisms, the concentrations are given which produce a 50% mortality or growth inhibition response at acute exposure, and cause no harmful effect in chronic studies.

A safety factor is derived on the basis of the scatter in these concentrations. The mean concentration is divided by the safety factor and the result is a safe limit for the most sensitive species (Kooijman, 1986).

The method of Slooff et al. (1986) derives a safe concentration for the most sensitive species from the experimental observations as follows: the concentration without effect on the most sensitive species (= the lowest concentration found) is divided by an uncertainty factor. This uncertainty factor is based on the residual standard deviation in the regression of the acute LC50 or EC50 value at to the no-effect concentration in chronic studies or in ecosystems for a large number of substances and species of organisms, reported in the literature.

THE SCATTER IN THE CONCENTRATIONS CORRESPONDING WITH A PARTICULAR RESPONSE OR EFFECT

Application of the safety factor and the uncertainty factor would certainly be useful if the scatter in the above-mentioned concentrations were based purely on differences in sensitivity among species. However, this is by no means the case as the scatter is strongly influenced by:

- differences in toxicity mechanism;
- differences in metabolism (degradation, bioaccumulation);

- differences in the test medium and the accompanying stress on the test organism;
- differences in test duration, expressed in the generation time of the test organism.

A growth-inhibition test for algae and bacteria is in fact a chronic test, but was regarded as an acute test in the derivation of the safety factor and the uncertainty factor.

The size of the safety factor (Kooijman, 1986) and the uncertainty factor (Slooff et al., 1986) is thus determined not only by differences in sensitivity among species but also by many other differences, as mentioned above.

The ill-considered use of these factors in the determination of safe limits for the so-called most sensitive species consequently results in extremely low limits, which have no ecotoxicological but purely a statistical significance.

CONCLUSION

As long as the safety factor and the uncertainty factor are not solely based on differences in sensitivity among the species of organisms, it is scientifically unacceptable to use these factors for deriving limits as described in the integrated criteria documents of the RIVM.

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TNO-report P 85/0426 dd. 1986-05-23

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EFFECTS

March 1989

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1. HUMAN TOXICITY - NITRATE AND NITRITE

1.1 Chemobiokinetics and metabolism

1.1.1 Animal studies - nitrate

Absorption

Ingested nitrate is primarily absorbed from the rat upper small intestine but hardly or not at all from the stomach and lower intestine (terminal ileum, caecum and proximal colon). Absorption is rapid: 50% of the radioactive label could be detected in the eviscerated rat carcass within 1 hour after oral gavage of $^{13}\text{NO}_3^-$ (Witter et al., 1979 a,b; Balish et al., 1981).

In cattle, nitrate was shown to be absorbed from the rumen. Peak blood nitrate levels occurred 4 hours after intraruminal gavage. When the same amount of nitrate was fed on hay, absorption was slower due to the lower rate of uptake and blood nitrate levels remained substantially lower. The extent of uptake did not change (Wright and Davison, 1964).

Uptake of nitrate from the upper respiratory tract was demonstrated to occur within 5 minutes after intratracheal administration in mice and rabbits (Parks et al., 1981).

Distribution

Low levels of nitrate are normally present in body fluids and tissues of laboratory animals (Witter and Balish, 1979; Fritsch et al., 1985). Normal plasma nitrate levels in mongrel dogs were 6-10 mg/l (= 0.1-0.15 mM; Fritsch et al. (1985)).

Nitrate from the blood is selectively distributed to the salivary glands and secreted in saliva in most laboratory animals but not in rats (Fritsch et al., 1985; Nighat et al., 1981; Witter and Balish, 1979). The transport of nitrate from blood to saliva is probably competitively mediated by active carriers that are shared by a.o. iodide and thiocyanate (SCN^-) (NAS, 1981), but in rats this active transport system is lacking (as cited from Cohen and Myant (1959) by Mirvish (1983)).

Absorbed nitrate may re-enter the stomach and intestinal lumen directly via the bloodstream and via secretions. Secretion of nitrate into the stomach

may be mediated by active carriers similar to those in the salivary glands (cited by Edwards et al., 1954). Nitrate in the lower intestine of rats was demonstrated to originate directly from blood or intestinal secretions rather than from the passage of gastric contents or secretions of bile and pancreatic juice (Witter et al., 1979a). However, rats may be exceptional in this respect as they are able to excrete iodide into the small intestine whereas this is unlikely to occur in other animal species (cited from Acland and Illman (1959) and Brown-Grant (1961) by the NAS, 1981).

After intravenous injections of ^{13}N -labelled nitrate into mice and rabbits, an equilibrium with extracellular fluid was obtained within 5 minutes (Parks et al., 1981).

Intratracheal instillation of ^{13}N -labelled nitrate in mice and rabbits gave very similar results (Parks et al., 1981).

After 20 minutes, 16% of the radioactivity from intravenously injected $^{13}\text{NO}_3^-$ could be detected in the stomach and intestines of rats, 7% was found in the liver, kidney and bladder, and 70% was present in the eviscerated carcass (Witter et al., 1979b). Salivary glands were not examined.

Nitrate is transported into milk. Nitrate levels in milk of lactating rats and cows given high nitrate doses were elevated (Ariga et al., 1984; Nijhuis et al., 1982). Nitrate is also frequently detected in normal cows' milk (NAS, 1981). However, the nitrate concentration in milk did not exceed the plasma nitrate level in a beagle dog after intravenous nitrate injection (Green et al., 1982) indicating that unlike salivary secretion, nitrate transport into milk is not an active process.

Biotransformation

The most important metabolite of nitrate is nitrite. However, nitrite is converted rapidly -see 1.1.2 biotransformation- and may not be readily detectable. Therefore, methaemoglobin formation (which is caused by nitrite, see 1.1.2 methaemoglobin formation) is often used as an indicator for nitrite formation although it may not be a very sensitive measure (Ward et al., 1986).

Part of ingested nitrate in CV (conventional flora) rats -but not in GF (germ-free) rats- was reported to be metabolized to NH_4^+ and urea (Green et al., 1981; Schultz et al., 1985).

Bacterial reduction is an important mechanism for nitrate conversion in mammals (Witter and Balish, 1979; Green et al., 1981; Schultz et al.,

1985). Nitrate reductase is present in many bacteria and other micro-organisms normally occurring in the gastro-intestinal tract (WHO, 1985).

Turek et al. (1980) observed that nitrate reduction by the fecal flora of pigs under anaerobic conditions was more rapid after a prolonged high-nitrate diet, suggesting the possibility of bacterial selection or induction. Wise et al. (1982) observed a several-fold increase in nitrite production in the rat caecum when adding 5% pectin to the diet. According to the authors, this increase could not be attributed to overall differences in the diversity or number of micro-organisms, which is in favour of the possibility of bacterial enzyme induction.

Nitrate reductase activity has also been demonstrated in various rat tissues (WHO, 1985; Ward et al., 1986). Schultz et al. (1985) detected that, in rats, 90% of total mammalian tissue nitrate reductase activity was present in the liver.

The same authors calculated from the urinary nitrate excretion in CV and GF rats after intraperitoneal nitrate injection that approximately half of the metabolized nitrate in CV rats (=20-23% of total injected nitrate; 40-45% is metabolized) was metabolized by mammalian processes and the other half by enteric bacteria.

In the ferret, 67% of a large single oral $^{15}\text{NO}_3^-$ dose was metabolized (Dull and Hotchkiss, 1984). The ferret may be a more suitable experimental animal than the rat because its basal stomach acidity and gastric morphology are more similar to those of humans.

Nitrate reduction in man, and probably in most animal species, takes place for the largest part in the oral cavity (saliva). It may also occur throughout the gastro-intestinal tract; however, the conversion is pH-dependent and therefore does not occur in the stomach of most monogastric animals (Wright and Davison, 1964; Mirvish et al., 1975).

The rumen of ruminants and the enlarged caecum and colon of horses are especially suited for nitrate reduction due to the dense microbial population and the relatively high pH (Wright and Davison, 1964).

In rabbits and ferrets, the average gastric pH is low and therefore considered to be similar to that in man (Sen et al., 1969; Dull and Hotchkiss, 1984); in cats, rats and dogs it is higher (2.9, 4-5 and 5.4-7.4, respectively - Sen et al., 1969). In GF rats, the pH of various parts of the gastro-intestinal tract is significantly higher than in CV rats (Ward et al., 1986).

Salivary nitrate reduction is almost absent in rats (Witter et al., 1979a; Til, 1986) which is probably due to the low salivary nitrate secretion in this species.

Excretion

Nitrate excretion in urine generally reflects nitrate intake. However, various authors have reported that urinary nitrate excretion may exceed nitrate intake if the latter is low, as a consequence of endogenous nitrate formation, see 1.1.1 biosynthesis.

In CV rats, approximately 55% of orally administered ^{15}N -labelled nitrate was excreted unaltered into urine. 11% Of the label was detected as urinary ammonia and urea (Schultz et al., 1985). Nitrate is hardly or not excreted into faeces of CV and GF rats (Green et al., 1981), which leaves 34% unaccounted for (Schultz et al., 1985).

In ferrets, urinary nitrate excretion amounted to 36% of an ingested dose. Other nitrogen compounds were not monitored (Dull and Hotchkiss, 1984).

Fritsch et al. (1985) found that nitrate could be excreted in the saliva and bile of dogs in concentrations similar to plasma nitrate values.

The elimination of nitrate from plasma was demonstrated to vary considerably between species. The elimination half-life of nitrate resulting from nitrite injection -see 1.1.2 oxidation- was 45 hours for dogs, whereas for sheep and ponies it was only 4 hours (Schneider and Yeary, 1975).

Biosynthesis

If nitrate intake is low, urinary nitrate excretion usually exceeds intake. This was demonstrated by Green et al. (1981) to be the case in CV and GF rats. This suggested that nitrate was newly synthesized in the animals. Furthermore, the higher excretion in GF as well as in CV rats showed that bacterial activity was not obligatory for this synthesis.

The inhalation of nitrogenous oxides from the air could account for, at most, 1% of the excess excreted nitrate (Green et al., 1981).

Actual proof of nitrate biosynthesis was supplied by Dull and Hotchkiss (1984) for ferrets and by Saul and Archer (1984) and Wagner et al. (1983a) for rats. Ingestion of ^{15}N -labelled ammonium salts was invariably followed by the urinary excretion of small amounts of ^{15}N -labelled nitrate.

It was proposed that ammonia is at first oxidized to hydroxylamine, catalyzed by the generation of free radicals, which is then further oxidized to

yield nitrate (Wagner et al., 1983a). This hypothesis was confirmed by the experimental in vivo synthesis of nitrate from hydroxylamine and the enhanced synthesis of nitrate from ammonia by rats treated with an endotoxin inducing free radical formation (Saul and Archer, 1984; Wagner et al., 1983a). Wagner et al. (1983a) showed that urinary nitrate excretion increased 9 times after intraperitoneal injection of E. coli lipopolysaccharides, whereas the ;nitrate intake did not change. Stuer and Marletta (1985) found that infection with Mycobacterium bovis could increase the urinary nitrate excretion of mice from 3,6 to 164 mg/kg b.w. Both studies indicate that endogenous nitrate synthesis may increase considerably under inflammatory conditions.

1.1.2 Animal studies - nitrite

Absorption

Nitrite rapidly disappeared from a buffered solution at pH lower than 5 (under simulated gastric conditions) and the rate of disappearance was enhanced by the presence of food (Mirvish et al., 1975). It may also react with gastric contents (e.g. nitrosation -WHO, 1985; see also 1.3 nitrosation-) or be reduced by the gastro-intestinal flora (see 1.1.2 reduction). A large part of nitrite entering the gastro-intestinal tract may thus have disappeared before absorption is due.

Absorption of nitrite-nitrogen from the gastro-intestinal tract of rats was slower than that of nitrate. 45 Minutes after intragastric instillation of ¹³N-labelled nitrite, radioactivity was higher in the stomach and lower in the organs (liver, kidneys and bladder) and eviscerated carcass than in similar experiments with ¹³N-labelled nitrate (Witter et al., 1979b).

Nitrite could not be absorbed from the caecum and large intestine of rats (Witter and Balish, 1979).

Gastric absorption of nitrite was noticed in labelling studies and gastric emptying studies using rats and mice (Witter et al., 1979b; Mirvish et al., 1975). In mice gastric absorption of nitrite seemed to be faster than in rats (Friedman et al., 1972).

Distribution

Nitrite is normally absent from the body fluids and tissues of laboratory animals (Witter and Balish, 1979; Fritsch et al., 1985).

Intravenous injections and intratracheal instillation of ^{13}N -labelled nitrite into mice and rabbits both resulted in homogenous distribution of radioactivity among heart, kidneys, liver, stomach, intestines, lungs and bladder within 5 minutes, similar to nitrate distribution. (Parks et al., 1981). 30 Minutes after intravenous injection of nitrite, only low levels of nitrite could be detected in blood and saliva of dogs (Fritsch et al., 1985).

Half-lives of nitrite plasma values in the distribution phase were 48 minutes for dogs, 12 minutes for sheep and 5 minutes for ponies (Schneider and Yeary, 1975).

Nitrite could cross the placenta of rats and guinea pigs: nitrite injected into pregnant animals appeared in fetal blood -in a lower concentration than in maternal blood- after a lag time of approximately 20 minutes (Grue-ner et al., 1973; Sinha and Sleight, 1971).

Transport of large amounts of nitrite into milk is unlikely: nitrite doses inducing methaemoglobinemia in nursing rats did not cause the same adverse effect in sucklings (Gruener et al., 1973).

Biotransformation

Reduction

CV rats receiving 1000 mg/l nitrite in drinking water had lower nitrite levels in gastric and intestinal tissues than GF rats, whereas nitrate levels were about equal. This indicated a similar uptake of nitrite in both groups but a faster conversion rate in CV rats. In vitro experiments confirmed that intestinal bacteria are involved in the reduction of nitrite (Balish et al., 1981; Witter and Balish, 1979).

Oxidation

Absorbed nitrite is rapidly oxidized to nitrate in the blood (Witter and Balish, 1979; Fritsch et al., 1985; Parks et al., 1981). This process is considered to be mammalian rather than bacterial (Witter and Balish, 1979). Intravenous injections of sodium nitrite (20 mg/kg b.w.) into dogs, sheep and ponies resulted in plasma nitrate levels of 40-100 mg/l (=0.6-1.5 mM) in all three species within minutes (Schneider and Yeary, 1975).

Nitrite oxidation to nitrate may also occur in the stomach prior to absorption, as was demonstrated in vitro for mice. However, under in vivo conditions nitrite is probably absorbed from the stomach before large quantities

of nitrate have been formed (Friedman et al., 1972).

Methaemoglobin formation

Nitrite is involved in the oxydation of haemoglobin (Hb), which is normally present in blood, to methaemoglobin (methHb). The Fe^{2+} atom present in the haem group is oxidized to its Fe^{3+} form and nitrite firmly binds to this oxidized haem. Thus, blood oxygen transport is inhibited (Jaffé, 1981).

The formation rate of methHb varies considerably between species. Hb solutions of ruminants (sheep, goat, cow) incubated with freshly prepared sodium nitrite gave a much faster rate of methHb formation than that of man or horse, whereas for pigs this rate was slower (Smith and Beutler, 1966).

At low levels of nitrite, methHb formation is reversible; the rate of methHb reduction to normal Hb, which is catalyzed by the enzyme NADPH-methHb reductase, was demonstrated to vary also for different species, with a high correlation between methHb formation and reduction rate (Smith and Beutler, 1966).

Methylene blue has a protective effect against nitrite-induced methHb formation (Sheehy and Way, 1974) and may therefore be used as an antidote in nitrite intoxication; it is also used to verify whether certain toxic effects are mediated by nitrite and methHb or by other compounds, e.g. in nitrate feeding studies.

Dietary factors may have a protective effect against methHb formation: ascorbic acid and methionine added to the diet of guinea pigs reduced the level of nitrite-induced methHb, whereas vitamin C deficient guinea pigs had higher nitrite-induced methHb levels than control animals (various authors, cited in the WHO, 1977).

Dietary factors may also influence the effects of nitrate and nitrite in a negative way: high pectin intake stimulated nitrite formation from ingested nitrate and thus increased the formation of methHb in rats (Wise et al, 1982).

Reported reduced vitamin A liver deposits after nitrite intake are probably caused by direct reaction of nitrite with carotene prior to absorption (WHO, 1977; Emerick, 1974).

Excretion

Urinary and faecal excretion of nitrite are very low, as most of the nitrite that enters the bloodstream or passes down the gastro-intestinal tract

is rapidly converted to nitrate, bound to the gastro-intestinal contents, or reduced by enteric bacteria. Nitrite is not secreted into saliva or bile in significant amounts (Fritsch et al., 1985).

The elimination half-life of nitrite (metabolism plus urinary excretion) in dogs, sheep and ponies was similar and amounted to 0.5 hours (Schneider and Yeary, 1975).

1.1.3 Human studies - nitrate

Absorption

Nitrate is primarily absorbed from the upper part of the human digestive tract (Bartholomew and Hill, 1984; Witter et al., 1979a). Absorption is rapid: within 1-3 hours after ingestion of nitrate in food or drink peak levels of nitrate were observed in serum, saliva and urine by various investigators (Bartholomew and Hill, 1984; Ellen et al., 1982b; Spiegelhalder et al., 1976; Turek et al., 1980).

Distribution

Absorbed nitrate is rapidly distributed to the salivary glands and probably also to other exocrine glands. 1 To 3 hours after ingestion a peak value of nitrate could be observed in saliva and sweat and the increase in the amount of nitrate secreted by the salivary glands depended directly on the amount of nitrate ingested (Spiegelhalder et al., 1976; Bartholomew and Hill, 1984).

The transport of nitrate to the salivary glands is probably mediated by active carriers. Edwards et al. (1954) reported substrate inhibition of active iodide secretion into saliva by nitrate and also by thiocyanate (SCN^-) and perchlorate (ClO_4^-). Thus, SCN^- , iodide and ClO_4^- would also be able to inhibit nitrate secretion into saliva. This may be of importance for smokers, in whom SCN^- levels are 3 to 4 times higher than in non-smokers (cited by Boyland and Walker, 1974). Forman et al. (1985) actually found lower nitrate levels in the saliva of smokers than of non-smokers.

Salivary nitrate levels were found to be generally higher at higher adult age (Forman et al., 1985) However, salivary nitrate levels depend largely on nitrate intake; salivary concentrations reported for adults range from less than 0.1 mM after low nitrate intake (Turek et al., 1980). to over 40 mM after a high nitrate dose (Ellen et al., 1982b). Salivary nitrate levels

reported for a group of breast- and bottle-fed infants were 0.1-1.0 mM with an average of 0.5 mM (Turek et al., 1980).

After intravenous administration of ^{13}N -labelled nitrate into one volunteer, the label was rapidly distributed with the bloodstream throughout the body. The radioactivity accumulated almost linearly with time in a small region of the abdomen, which was probably due to the swallowing of salivary nitrate (Witter et al., 1979a).

Nitrate may be present in human milk. Levels of upto 5 mg NO_3^-/kg milk were reported (Sukegawa and Matsumoto, 1975). However, nitrate levels in milk from lactating women after a normal evening meal did not exceed the corresponding elevated plasma nitrate levels (Green et al., 1982, citation from unpublished results).

Biotransformation

Nitrate is partly converted to nitrite by micro-organisms in saliva. 4-7% Of ingested nitrate can be detected as nitrite in the saliva (Eisenbrand et al., 1980; Stephany and Schuller, 1980). The ratio nitrite/nitrate in saliva 1-2 hours after intake of various nitrate doses was remarkably constant within one person but differed greatly between persons (from 0.06 to 3.6, Bartholomew and Hill, 1984; Ellen et al., 1982b).

Factors that may influence the oral microbial status are, for example, nutritional status, infection, environmental temperature and age. A sudden drop of temperature resulted in a dramatic fall of salivary nitrite levels, but this may have been caused by increased salivary flow as well as by reduced microbial activity (Eisenbrand et al., 1980). Salivary nitrite levels were generally higher in higher age groups, although considerable variation between persons was noted. (Eisenbrand et al., 1980; Forman et al., 1985). A low pH (1-2) in the fasting stomach is considered normal for adults, and bacterial nitrate reduction does not take place at low pH due to poor bacterial growth.

High gastric pH values and sometimes corresponding high nitrite levels, however, were observed in stomach cancer and gastric ulcer patients, in patients with atrophic gastritis and in patients treated with cimetidine and antacids (Correa et al., 1975; Ruddell et al., 1976; Schlag et al., 1982; Bartsch et al., 1984).

Infants younger than 3 months are also highly susceptible to gastric bacterial nitrate reduction because they have very little production of gastric

acid (as cited in a review by Ellen and Schuller, 1983). Gastrointestinal infections, which frequently occur in infants, may give an additional increase in nitrate reduction to nitrite.

Contrary to the usual assumption of a low normal gastric pH, Ruddell et al. (1976) and Mueller et al. (1984) found that a high proportion of normal healthy adults (30-40%) had a fasting gastric pH of higher than 5, which was relatively stable over a prolonged period, with corresponding high bacterial activity and high nitrite levels. In one third of 15 healthy volunteers major variations of the fasting gastric pH occurred occasionally, with corresponding changes in the bacteriological parameters (Mueller et al., 1984).

Schultz et al. (1985) developed a model for the fate of nitrate in humans based on various human data which was tested in rats. The model suggests that the bacteria of the large intestine are responsible for about half of the extrarenal removal of nitrate from the body.

Ascorbic acid affected neither the amounts of nitrate excreted in urine, faeces and saliva, nor nitrate plasma levels, indicating that it does not interfere with nitrate metabolism (Wagner et al., 1983b).

The half-life of nitrate in the body after ingestion was found to be approximately 5 hours (Wagner et al., 1983b).

Excretion

Single oral doses of 25-170 mg potassium nitrate gave a urinary nitrate excretion of 65-70% irrespective of the dose. Excretion was maximal 5 hours after ingestion and returned to baseline levels within 18 hours. Reported urinary nitrate baseline levels in fasting subjects were 10-20 mg/l (Bartholomew and Hill, 1984). Similar results were obtained by Tannenbaum and Green (1981) and Wagner et al. (1983b).

Wagner et al. (1983b) also found small amounts of ^{15}N -labelled ammonia and urea in the urine after ingestion of ^{15}N -labelled nitrate.

Larger single oral doses (7-10.5 g) of ammonium nitrate resulted in an average urinary nitrate excretion of 75% within 24 hours, whereas small amounts of nitrite could be detected in only 26% of the samples. In this study, nitrate baseline levels in urine were higher (2.4-9.3 mmol/l = 149-577 mg/l), probably because the subjects were not submitted to dietary restrictions (Ellen et al., 1982b).

In one study, urinary nitrate excretion in infants was reported to be 80-

100% of the average intake, but no specific data were given for uptake (Turek et al., 1980).

In another study with healthy infants, the urinary excretion of nitrate (3-16 mg, average 8.7 mg NO_3^- per day) was as high or higher than the average (low) intake of 2-7 mg NO_3^- plus NO_2^- per day (Hegesh en Shiloah, 1982), and it was concluded that excretion probably included endogenously synthesized nitrate.

Low levels of nitrate and nitrite were detected in the faeces of humans on a free Western diet with unknown nitrate content (Saul et al., 1981) and less than 0.1% ^{15}N -labelled nitrate was found in the faeces of 12 volunteers ingesting 298 mg of ^{15}N -labelled sodium nitrate. ^{15}N -Labelled ammonia and urea were also detected in small quantities (Wagner et al., 1983b).

Incubation of nitrate with fresh human faeces under anaerobic conditions resulted in a rapid conversion of nitrate by the faecal microflora, suggesting that real faecal excretion of nitrate may be higher than can be detected (Archer et al., 1981; Saul et al., 1981).

Biosynthesis

Various authors reported an excess nitrate excretion (0.3-1.9 mmol/day urinary excretion) at low nitrate intake (less than 0.25 mmol/day) in humans (Bartholomew and Hill, 1984; Green et al., 1981b; Lee et al., 1986; Tannenbaum and Green, 1981; Wagner et al., 1983b). If large amounts of ^{15}N -labelled nitrate were ingested (up to 3.5 mmol), the urinary excretion of unlabelled nitrate was still considerable (0.7-1.3 mmol against 0.2 mmol ingestion - Green et al., 1981b).

Ellen and Schuller (1983) calculated that up to 20% of this excess could have originated from the inhalation of NO_2^- and NO_3^- from indoor and outdoor air and cigarette smoke.

The remaining excess urinary nitrate, up to 1 mmol/day, very probably originates from endogenous synthesis. Direct proof of in vivo nitrate synthesis from ammonia and hydroxylamine has been given in rats and ferrets - see 1.1.1 biosynthesis. Although bacterial activity is not obligatory for this synthesis in animals, bacterial activity may enhance nitrate biosynthesis; the occurrence of gastrointestinal infections may be of importance in this matter. Hegesh en Shiloah (1982) found a considerably increased urinary nitrate excretion in infants with acute diarrhea (from 8.7 to 39 mg NO^- per 24 hours), at a low intake of 2 to 7 mg NO_3^- plus NO_2^- daily.

1.1.4 Human studies - nitrite

Absorption

No data concerning the absorption of nitrite in man were found. However, as part of ingested nitrate is converted to nitrite in the oral cavity and the stomach, and since the occurrence of high methHb levels in infants ingesting large nitrate doses have been reported (Shuval and Gruener, 1972), nitrite may obviously be absorbed from the gastro-intestinal tract.

Distribution

No data concerning the distribution of nitrite in the human body were found.

Biotransformation

Reduction

Nitrite reduction in vivo, yielding nitric oxide (NO), may be catalyzed by nutritional components like ascorbic acid and alpha-tocopherol (Newmark and Mergens, 1981).

Oxidation

No data were found concerning the in vivo oxidation of nitrite to nitrate in man.

Methaemoglobin formation

Like in animals, nitrite may give rise to the formation of methHb in humans. The rate of methHb formation as well as of methHb reduction for humans is slower than for ruminants, but faster than for horses and pigs (Smith and Beutler, 1966).

Infants are extremely susceptible to nitrite-induced methHb formation (reviewed by Ellen and Schuller, 1983):

- fetal Hb, which comprises initially 60-80% of total Hb and decreases to 20-30% within 3 months, is more easily converted to methHb
- NADPH-methHb reductase, which catalyzes methHb reduction back to Hb, is normally deficient in neonatal erythrocytes

Excretion

Archer et al. (1981) and Saul et al. (1981) detected low levels of nitrite in the faeces of humans on a free Western diet with unknown nitrate and nitrite content. Like nitrate, nitrite incubated with fresh human faeces un-

der anaerobic conditions was rapidly converted by the faecal microflora, suggesting that actual nitrite excretion may well be higher than can be detected.

1.1.5 Summary

Nitrate is primarily absorbed from the upper part of the digestive tract in humans as well as in rats (Bartholomew and Hill, 1984; Witter et al., 1979a). Absorption is rapid: within 1-3 hours after ingestion of nitrate in food or drink peak levels of nitrate were observed in human serum, saliva and urine by various investigators (Bartholomew and Hill, 1984; Ellen et al., 1982b; Spiegelhalder et al., 1976; Turek et al., 1980).

Low levels of nitrate are normally present in body fluids and tissues (Witter and Balish, 1979; Fritsch et al., 1985).

Nitrate from the blood is selectively distributed to the salivary glands and secreted in saliva in humans and in most laboratory animals, but not in rats. The transport of nitrate from blood to saliva is probably competitively mediated by active carriers that are shared by a.o. iodide and thiocyanate (SCN^-) (NAS, 1981), but in rats this active transport system is lacking (as cited from Cohen and Myant (1959) by Mirvish (1983)).

Nitrate may be transported into milk, but unlike salivary secretion, nitrate transport into milk is not an active process (Green et al., 1982).

Nitrate is partly converted to nitrite by micro-organisms in saliva. 4-7% ingested nitrate can be detected as nitrite in the saliva (Eisenbrand et al., 1980; Stephany and Schuller, 1980).

Bacterial nitrate reduction does not take place at low pH due to poor bacterial growth and is therefore considered not to take place in the stomach of most monogastric animals including man (Wright and Davison, 1964; Mirvish et al., 1975). High gastric pH values and sometimes corresponding high nitrite levels, however, were observed in stomach cancer and gastric ulcer patients, in patients with atrophic gastritis and in patients treated with cimetidine and other antacids (Correa et al., 1975; Ruddell et al., 1976; Schlag et al., 1982; Bartsch et al., 1984). Infants younger than 3 months are also highly susceptible to gastric bacterial nitrate reduction because they have very little production of gastric acid (as cited in a review by Ellen and Schuller, 1983). Gastrointestinal infections, which frequently occur in infants, may give an additional increase in nitrate reduction to nitrite.

Salivary nitrate reduction is almost absent in rats (Witter et al., 1979a; Til, 1986) which is probably due to the low salivary nitrate secretion in this species.

The half-life of nitrate in human body after ingestion was found to be approximately 5 hours (Wagner et al., 1983b).

Single oral doses of 25-170 mg potassium nitrate gave a urinary nitrate excretion of 65-70% in humans irrespective of the dose. Excretion was maximal 5 hours after ingestion and returned to baseline levels within 18 hours (Bartholomew and Hill, 1984). Larger single oral doses (7-10.5 g) of ammonium nitrate resulted in an average urinary nitrate excretion of 75% within 24 hours, whereas small amounts of nitrite could be detected in 26% of the samples (Ellen et al., 1982). In one study with infants, urinary nitrate excretion was reported to be 80-100% of the average intake, but no specific data were given (Turek et al., 1980), but another study with infants showed that nitrate excretion at low intake may include endogenously synthesized nitrate (Hegesh and Shiloah, 1982).

Various authors reported an excess nitrate excretion (0.3-1.9 mmol/day urinary excretion) at low nitrate intake (less than 0.25 mmol/day) in humans (Bartholomew and Hill, 1984; Green et al., 1981b; Lee et al., 1986; Tannenbaum and Green, 1981; Wagner et al., 1983b). If large amounts of ^{15}N -labelled nitrate were ingested (up to 3.5 mmol), the urinary excretion of unlabelled nitrate was still considerable (0.7-1.3 mmol against 0.2 mmol ingestion - Green et al., 1981b). Ellen and Schuller (1983) calculated that up to 20% of this excess could have originated from the inhalation of NO_2^- and NO_3^- from indoor and outdoor air and cigarette smoke. The remaining excess urinary nitrate, up to 1 mmol/day, very probably originates from endogenous synthesis. Actual proof of nitrate biosynthesis was supplied by Dull and Hotchkiss (1984) for ferrets and by Saul and Archer (1984) and Wagner et al. (1983a) for rats. Ingestion of ^{15}N -labelled ammonium salts was invariably followed by the urinary excretion of small amounts of ^{15}N -labelled nitrate. Although bacterial activity is not obligatory for this synthesis in animals, bacterial activity may enhance nitrate biosynthesis. The occurrence of gastrointestinal infections may thus increase nitrate biosynthesis considerably (Wagner et al., 1983a; Hegesh and Shiloah, 1982; Stuerer and Marletta, 1985).

A large part of nitrite entering the gastro-intestinal tract may have dis-

appeared before absorption is due, as a result of reaction with gastric contents (e.g. nitrosation -WHO, 1985; see also 1.4 Nitrosation-) or reduction by the gastro-intestinal flora.

Gastric absorption of nitrite was noticed in labelling studies and gastric emptying studies using rats and mice (Witter et al., 1979b; Mirvish et al., 1975). No data concerning the absorption of nitrite in man were found. However, as part of ingested nitrate is converted to nitrite in the oral cavity and the stomach, and since the occurrence of high methHb levels in infants ingesting large nitrate doses have been reported (Shuval and Gruener, 1972), nitrite may obviously be absorbed from the gastro-intestinal tract. Nitrite may be reduced in the gastro-intestinal tract, yielding nitric oxide (NO) (Newmark and Mergens, 1981).

Absorbed nitrite is rapidly oxidized to nitrate in the blood (Witter and Balish, 1979; Fritsch et al., 1985; Parks et al., 1981). This process is considered to be mammalian rather than bacterial (Witter and Balish, 1979). Nitrite oxidation to nitrate may also occur in the stomach prior to absorption, as was demonstrated in vitro for mice. However, under in vivo conditions nitrite is probably absorbed from the stomach before large quantities of nitrate have been formed (Friedman et al., 1972).

Nitrite in the blood stream (exogenous nitrite as well as nitrite formed out of exogenous and/or endogenously synthesized nitrate) is also involved in the oxydation of haemoglobin (Hb) to methaemoglobin (methHb). The Fe^{2+} atom present in the haem group is oxidized to its Fe^{3+} form and nitrite firmly binds to this oxidized haem. Thus, blood oxygen transport is inhibited (Jaffé, 1981). At low levels of nitrite, methHb formation is reversible.

Infants are extremely susceptible to nitrite-induced methHb formation (reviewed by Ellen and Schuller, 1983) because:

- fetal Hb, which comprises initially 60-80% of total Hb and decreases to 20-30% within 3 months, is more easily converted to methHb.
- NADPH-methHb reductase, which catalyzes methHb reduction back to Hb, is normally deficient in neonatal erythrocytes.

The fate of nitrate and nitrite in the mammalian body is schematically illustrated in Figure I.

1.2 Animal experiments

1.2.1 Nitrate

Acute toxicity

The oral LD50 values for mice varied from 2480 to 6250 mg sodium nitrate/kg body weight. The oral LD50 for rats varied from 4860 to 9000 mg sodium nitrate/kg b.w. Female rats seemed to be more sensitive than males (Toxic Substances List, 1980; Mamm. Toxicity Array, 1982; Corr  and Breimer, 1979). The LD50 for rabbits is 1600 mg sodium nitrate/kg body weight. For pigs a lethal dose of 300 mg sodium nitrate/kg b.w. has been reported. The oral LD50 for cows after a single administration was estimated to be 450 mg sodium nitrate/kg b.w. (Bradley et al., 1942), whereas the LD50 was 970-1360 mg per kg b.w. (Crawford, 1960), when the total dose was administered over a 24 hours period. The lethal dose for cows appeared to be ten times lower than for non-ruminants (Gwatkin and Plummer, 1946; Emerick, 1974). Acute intoxication occurred in cattle when rations with 6% or more nitrate in dry matter were fed. Fatal intoxications in cattle were also reported at dose levels of 1.5% in feed (Bradley et al., 1940), whereas hay with 0.75% nitrate as dry matter revealed no adverse effects (Geurink and Kemp, 1983).

Subacute toxicity

(Data of the experiments are detailed in Table II.)

In a 4-week toxicity study rats (10/sex/group) were fed diets containing 0, 1, 2, 3, 4 or 6% potassium nitrate or 5% sodium nitrate (approximately 0, 500, 1000, 1500, 2000, 3000 or 2500 mg/kg b.w.). Two types of diet were used: a cereal basal and a semi-purified diet. At a dose level of 2% potassium nitrate (1000 mg/kg b.w.) in the diet the female rats revealed slightly elevated methaemoglobin levels and the male rats showed increased relative kidney weights. At the dose level of 1% (500 mg/kg b.w.) no toxic effects were observed. No important differences were found between the two types of diets (Til et al., 1985a,b).

Subacute toxicity studies with F344 rats (10/sex/group) fed a diet containing 1.25, 2.5, 5, 10 and 20% sodium nitrate (approximately 625, 1250, 2500, 5000 and 10000 mg/kg b.w.) for 6 weeks revealed a slight or moderate reduced weight gain at the two highest dose levels, and at autopsy an ab-

normal colour of the blood and spleen, due to methaemoglobinemia. The maximum tolerated dose was 5% in the diet (Maekawa et al., 1982).

In a 4-week study rabbits (6 males/group) received 0, 200, 400 and 600 mg potassium nitrate/kg b.w. in a pulse dose via gelatin capsules. The rabbits of all nitrate treatment groups did show intoxication symptoms within two weeks including significant weight reduction, tachycardia, polyuria and weakness (Nighat et al., 1981).

In a subacute toxicity experiment 3 dogs (2 females, 1 male) were fed a diet containing 2% sodium nitrate (approximately 500 mg/kg b.w.) for 105 and 125 days. No adverse effects were noticed (Lehman, 1958).

In an 8-week toxicity study calves (12 males/group) received artificial milk containing 18 (control group), 400, 2000, 5000 and 10000 mg NO_3^- /kg. No adverse effects were observed on growth pattern, weight gain, food conversion, the biochemical blood parameters, and morphology of liver and kidneys (Berende et al., 1977).

Semichronic toxicity

(Data of the experiments are detailed in Table II.)

In a semichronic toxicity study (12 weeks) rats were administered 0 or 1/20 of the LD50 of sodium or calcium nitrate by gastric intubation. In the nitrate group the energy conversion processes were reported to be altered, such as the glycolysis and the pentose phosphate cycle, and changes in the glutathione-ascorbinate system of liver and brain tissue were reported (Diskalenko et al., 1972a and b; cited in WHO, 1978).

In a 14 month toxicity experiment rats (10/sex/group) received drinking water containing 0 or 4000 mg sodium nitrate/l (approximately 0 or 400 mg/kg b.w.). The methaemoglobin levels in the nitrate group were the same as for the control animals. Nitrate had a moderate effect on plasma vitamin E level and the incidence of chronic pneumonitis (Chow et al., 1980).

Long-term toxicity

(Data of the experiments are detailed in Table III.)

In a 2 year experiment rats (20/sex/group) were fed a diet containing 0, 0.1, 1, 5 and 10% (approximately) sodium nitrate. At the 5% dose level a slight growth inhibition was observed, whereas inanition was noticed at the 10% dose level. Complete histopathological examination, including tumour incidences, was performed. No further abnormalities or increase in tumours

were found. The no toxic effect level in this study was 1% (or 500 mg/kg b.w.) (Lehman, 1958; WHO, 1962; 1974).

Carcinogenicity

(Data of the experiments are detailed in Table III.)

In a feeding study of more than 1 year mice (10/sex/group) received a diet containing 0, 25000 or 50000 mg sodium nitrate/kg. In these animals no difference in tumor incidences were observed (Greenblatt et al., 1973; Sugiyama et al., 1979, abstract only).

In a carcinogenicity experiment rats (15/sex/group) received 0 or 0.5% sodium nitrate/l drinking water (approximately 500 mg/kg b.w.) for 84 weeks and were killed 20 weeks later. Histopathological examination did not reveal any increase in tumor incidence (Lijinsky et al., 1973a). In another carcinogenicity experiment (2 year) with F-344 rats (50/sex/group) receiving drinking water containing concentrations of 0, 2.5 or 5.0% sodium nitrate (corresponding to about 0, 2500 or 5000 mg/kg b.w.) also no carcinogenic effects were detected. In the experimental groups a reduction was found in the incidence of mononuclear cell leukemias, which has a high frequency in control animals of this strain (Maekawa et al., 1982). This study was considered adequate.

Reproduction/fetotoxicity

(Data of the experiments are detailed in Table III.)

In a reproduction experiment with guinea pigs (4 (control), 6 (300) and 3 females/other groups) concentrations of 0, 300, 2500, 10000 and 30000 mg potassium nitrate/l drinking water were tested for 143-204 days. These doses corresponded to 0, 12, 102, 507 and 1130 mg potassium nitrate/kg b.w.. The mating behaviour was highly impaired at 30000 mg/l dose level and the number of pregnant animals was seriously reduced. The fertility of the animals of other nitrate groups appeared not to be reduced since pregnancy did occur in all groups. Weight gain and food and water intake were normal at all concentrations. No macroscopic or microscopic alterations were observed in the reproductive organs (Sleight and Atallah, 1968).

In a 7 months experiment 15 heifers were fed a diet containing 445 and 665 mg NO_3^- /kg from 2 months pregnancy until birth. No treatment-related changes in pregnancy were observed, although the dose levels selected led to 20-50% methaemoglobinemia. Macroscopical examinations revealed no abnor-

malities in the newborn animals (Winter and Hokanson, 1964).

In a study with 25 sheep (5/group), from day 21-49 of pregnancy till parturition fed a diet containing 0.3, 0.6 and 1.2% NO_3^- in drinking water (for a total of 41-74 days), a dose high enough to induce severe methaemoglobinemia, no changes in abortion rates were observed (Davison et al., 1965).

Mutagenicity

Nitrate induced no mutagenic effects in bacterial tests with Salmonella typhimurium; when tested under aerobic and anaerobic conditions in Escherichia coli mutagenicity was only found under anaerobic circumstances. The mutations, however, were probably due to reduction of nitrate to nitrite under these conditions (Konetzka, 1974).

In an in vitro chromosome aberration test with hamster cells sodium nitrate revealed mutagenic effects, whereas with potassium nitrate negative results were obtained. Sodium chloride - in contrast to potassium chloride - was also positive at high concentrations in the same test system (Ishidate et al., 1984). This made it likely that interactions may have taken place between the chromosomes and elevated concentrations of the sodium ions which subsequently could lead to chromosome aberrations (Ashby, personal communication).

Oral application of 500 mg sodium nitrate/kg b.w. to 11 or 12 days pregnant Syrian hamsters did not lead to an increase in gene mutations, chromosome abnormalities, micronuclei or morphological transformation in cells cultured from the hamster embryos (Inui et al., 1979).

In acute experiments mice were treated intragastrically with doses of 78.5, 235.5, 707 and 2120 mg sodium nitrate/kg b.w.. An increase in chromosome aberrations was found at only one dose (707 mg/kg b.w.) and the number of micronuclei was enhanced at 78.5 and 235.5 mg/kg b.w.. From the dose of 707 mg/kg onwards cytotoxicity occurred in the bone marrow as shown by a concomitant depression of the bone marrow.

In contrast, acute treatment of rats with doses up to 2120 mg/kg b.w. did not show chromosome abnormalities in the bone marrow. However, rats subacutely treated with the same doses of sodium nitrate, showed a significantly enhanced number of chromosome aberrations in bone marrow. According to the authors, however, it cannot be excluded that formation of N-nitroso compounds is responsible for the bone marrow damage (Luca et al., 1985).

1.2.2 Nitrite

Acute toxicity

The oral LD50 values for mice varied from 175 to 220 mg sodium nitrite/kg b.w. (Greenberg, 1945; Lehman, 1958) and the oral LD50 value for rats was 85 mg/kg b.w. (Lehman, 1958). The acute toxic effects of nitrite consisted of vasodilatation, lowering of the blood pressure, decrease in vitamin A content in the liver, and functional disturbances of the thyroid gland.

Dogs administered a single dose of 1-2 g sodium nitrite/kg sausage (further details not given) showed an increased respiration and heart rate, changes of the electrocardiogram (ECG), methaemoglobinemia within 1-2 hours, increased concentration of sodium, decreased concentration of potassium and increased aspartate amino transferase (ASAT) activity in serum (WHO, 1974).

A difference in toxicity was noticed in a special study with female rats in which a single dose was compared with a more continuous administration of the same dose. Using methaemoglobinemia as a parameter, a dose of 160 or 320 mg sodium nitrite/kg b.w. divided into smaller doses over a certain period (3 intervals of 15 min., followed by 4 intervals of 30 min.) appeared to be less toxic than a single dose of 40 or 80 mg/kg b.w. (De Vries, 1983).

In another acute experiment two doses of 100 mg sodium nitrite/kg b.w. administered with an interval of two hours to rats caused a high mortality, whereas all animals survived at an interval of 4 hours (Druckrey, 1963a). This difference could be related to the elimination half-life of methaemoglobin which was reported to be 90 minutes in rats (Shuval and Grüner, 1972).

Subacute toxicity

(Data of the experiments are detailed in Table II.)

In a 7 day toxicity experiment with 6 or 55 weeks old mice (5/group) 0 or 110 mg sodium nitrite/kg b.w. administered by gastric intubation a decrease in the forced running distance, abnormalities in the ECG and ultramicroscopic changes of the heart muscle were noticed (Kinoshita et al., 1985).

In a 2 week experiment concentrations of 0, 100, 1000, 1500 and 2000 mg sodium nitrite/l drinking water induced decreased motoractivity in mice. The doses were approximately 0, 10, 100, 150 and 200 mg/kg b.w. (Grüner and Shuval, 1971).

In a 6 week toxicity study rats (10/sex/group) received 0, 0.06, 0.125, 0.25, 0.5 and 1% sodium nitrite via drinking water. The doses corresponded to approximately 0, 60, 125, 250, 500 and 1000 mg/kg b.w.. In the 1% group a moderate growth depression was observed. At autopsy a marked change in colour of the blood and spleen due to methaemoglobinemia was noticed in the two highest dose groups. The maximum tolerated dose was 0.25% in drinking water (Maekawa et al., 1982).

In a 2 month toxicity study with rats (8 males/group) receiving 0, 100, 300 and 2000 mg sodium nitrite/l drinking water abnormalities in the EEG's were mainly observed in the highest dose group and to a lesser extent in the other dose groups. The doses corresponded to about 0, 10, 30 and 200 mg/kg b.w. (Shuval and Grüner, 1972).

In two subacute toxicity experiments (2 weeks) chickens were fed a diet containing 0 or 0.4% potassium nitrite (about 0 or 400 mg/kg b.w.) or 0 and 18-60 mg/kg b.w. sodium nitrite, respectively. The animals of the nitrite group showed growth retardation and an enlarged thyroid gland and decreased vitamin A content in the liver despite the vitamin A rich diet (Sell and Roberts, 1963; Brüggemann and Tiews, 1964).

Semichronic toxicity

(Data of the experiments are detailed in Table III.)

In a semichronic toxicity study mice (10 males/group) received drinking water containing sodium nitrite in concentrations of 0, 100, 1000, 1500 and 2000 mg/l, which approximated 0, 10, 100, 150 and 200 mg/kg b.w.. The motoric activity of the treated animals, especially at the highest dose level, was decreased. Methaemoglobineamia was also noticed. The sedating effect in the mice was not associated with the methaemoglobineamia according to the authors (Behrooz et al., 1971).

In a semichronic (16 weeks) toxicity experiment 1 month old rats (8 males/group) received 0 or 200 mg sodium nitrite/l via the drinking water (0 or 20 mg/kg b.w.). The methaemoglobin levels in the treated animals ranged from 0.5-3.1%, in comparison with 0-1.2% for the control group. A higher incidence of pulmonary lesions was noticed in the treatment group. In a second experiment with 2 month old rats (12 in the nitrite and 9 in the control group), sodium nitrite was administered in doses of 0 or 2000 mg/l for 14 months. The methaemoglobin levels fluctuated from 1-35% in comparison to 0-1% in the control group. The animals of the nitrite group had

lower body and liver weights and revealed decreased vitamin E levels in serum, while the lungs of all animals exhibited severe lesions. Also higher red blood cell reduced glutathion levels were found (Chow et al., 1980).

Long-term toxicity

(Data of the experiments are detailed in Table III.)

In a number of animal species including rats, pigs, sheep, and poultry chronic nitrite intoxication was reported to induce vitamin A deficiency. The vitamin A contents in the liver are depleted in non-ruminants due to its degradation under acid conditions in the intestinal lumen (Emerick, 1974; Sell and Roberts, 1963).

In a long-term toxicity study rats (8 males/group) received drinking water containing 0, 100, 1000, 2000 and 3000 mg sodium nitrite/l for 2 years. No significant differences between the control and the treatment groups were noticed in growth, development, mortality and total haemoglobin levels. In the 1000, 2000 and 3000 mg sodium nitrite/l dose groups the methaemoglobin blood levels were significantly increased to 5, 12 and 22% of the total haemoglobin, respectively. The major histopathological changes were observed in the lungs and the heart. At the highest concentration focal degeneration and fibrosis of the heart muscle was observed. This dose level corresponded with 250-350 mg sodium nitrite/kg b.w. for 2 years. The observation that the coronary arteries were thin-walled and dilated in these aged animals, instead of thickened and narrowed as is usually seen at that age, was difficult to interpret. The changes in the lungs consisting of dilatation of the bronchi with infiltration of lymphocytes and emphysema were observed in rats receiving 1000, 2000 and 3000 mg sodium nitrite/l drinking water (Shuval and Grüner, 1972).

Carcinogenicity

(Data of the experiments are detailed in Table III.)

In the report of the National Academy of Science, U.S. (NAS, 1981) 21 studies with mice and rats concerning the possible carcinogenicity of nitrite were summarized (Birdsall, 1981). According to the authors, however, a number of these studies did not meet the accepted standards for an adequate evaluation of carcinogenicity, due to the short duration, inappropriate route of administration, or the study not being designed to test nitrite (see Table III). None of the studies reported, indicated any carcinogenic

effect of nitrite (NAS, 1981; Birdsall, 1981).

Until recently, the major part of information on the possible carcinogenicity of nitrite was obtained from data including tumor incidences in animals used as controls receiving nitrite only, in studies designed to investigate the carcinogenic effects of simultaneous administration of nitrite and a nitrosatable amine. These studies are mainly summarized in Table III and in 1.3.

In a carcinogenicity experiment mice (50/sex/group) received drinking water containing 0, 1000, 2500 and 5000 mg sodium nitrite/l for 18 months. These doses corresponded to 0, 200, 500 and 1000 mg/kg b.w.. No changes in tumor incidences were observed (Inai et al., 1979).

In a large-scale study of the FDA (Newberne, 1978; 1979) using 573 control rats and 1383 treated rats, nitrite was administered via diet or drinking water in concentrations of 0, 250, 500, 1000 or 2000 mg/kg (approximately 0, 25, 50, 100 or 200 mg/kg b.w.). Part of the animals was exposed during their entire life-span starting 5 days prenatal, whereas another part was treated from the age of weaning onwards. Two types of diet were used: a conventional laboratory animal chow, and a semi-synthetic diet based on agar. Newberne reported an increase in incidence of lymphomas in all nitrite-treated groups (10.2% versus 5.4% in the control rats). A U.S. Governmental Interagency Working Group, however, came to different conclusions based upon examination of the same histological preparations. They diagnosed only a small number of lesions as lymphomas and assessed an incidence of approximately 1% in both treated and control groups. This discrepancy concerned the differentiation between the lymphomas as claimed by Newberne, and the extra-medullar haemotopoiesis, plasmacytosis or histiocytic sarcomas claimed by the Interagency Panel. Incidences of other types of tumors were also not increased (FDA, 1980a, b).

In a carcinogenicity experiment with F-344 rats (50/sex/group) receiving drinking water with a concentration of 0, 0.125 or 0.25% sodium nitrite for 2 years no carcinogenic effects were observed. A significant decrease in tumour incidence was found in the high dose females given nitrite, as compared to controls. Part of this decrease was accounted for by mononuclear cell leukaemias of which the spontaneous frequency is rather high (about 25%) in this strain (Maekawa et al., 1982).

In an experiment with F-344 rats (24/sex/group) receiving 2000 mg sodium nitrite/kg in feed or per l in drinking water, no carcinogenic effects were

found. The doses used corresponded to about 100 and 200 mg/kg b.w.. A decrease in monocytic leukaemia (a very common spontaneous neoplasm in F-344 rats) was observed in the treated groups in both sexes. Other types of tumours were not increased, neither in the animals fed nitrite in diet, nor in drinking water (Lijinsky et al., 1983).

In a recent study 70 male and 140 female F0 rats - divided into 6 groups - and their male and female offspring (F1) were fed cured meat containing 0, 200, 1000 and 4000 mg sodium nitrite/kg for their life-times. The canned pork meat was mixed in a ratio of 45% with a semi-synthetic diet, fed ad libitum. The daily exposure to sodium nitrite therefore was about 0, 5, 25 or 100 mg/kg b.w.. Reproduction was unaffected. No significant increase in tumour incidences were observed (Olsen et al., 1984). This study confirmed previous findings of Van Logten et al. (1972) who also studied rats (30/sex/group) fed a cured meat diet containing 0, 200 or 5000 mg sodium nitrite/kg. Here the canned meat was mixed in a weight ratio of 40% with a standard diet and fed first ad libitum, later on in rations of about 20 g/day. The daily dose of sodium nitrite therefore was about 0, 4 or 100 mg sodium nitrate/kg b.w..

Reproduction/fetotoxicity

(Data of the experiments are detailed in Table IV.)

In a fetotoxicity experiment with guinea pigs potassium nitrite was administered via the drinking water in concentrations of 300, 1000, 2000, 3000, 4000, 5000 and 10000 mg/l for 100-240 days. These doses correspond to 110, 270, 940, 1110, 1190, 1490 and 3520 mg/kg b.w., respectively. The number of female animals used for the different groups were: control: 4, 300, 1000 and 2000 mg: 3, 4000 mg: 6, 5000 mg: 4 and 10000 mg: 3. In each cage at least one male was present. Methaemoglobin-levels upto 20% were measured. In the 5000 or 10000 mg potassium nitrite/l dose groups 100% fetal mortality was recorded, whereas one of the females died. At the highest dose level growth inhibition in the maternal guinea pigs was observed. In animals with aborted, mummified or resorbed fetuses, inflammatory lesions of the uterus and cervix as well as degenerative lesions of the placenta were noticed. At the lower dose levels (300, 1000, 2000, 3000 and 4000 mg/l) no fetal mortality was observed (Sleight and Atallah, 1968).

In another fetotoxicity experiment, 4 pregnant guinea pigs/group received doses of 0, 50 or 60 mg sodium nitrite/kg b.w. by subcutaneous injection

during the last 15 days of pregnancy. In the 50 mg group the partus was normal, while one hour after administration of the 60 mg nitrite fetal death followed by abortion occurred in 3 animals. At that time, maternal and fetal blood methaemoglobin concentration had reached peak levels and oxygen pressure was lower in fetal blood than in the control animals. The dams in the 70 mg/kg group died within 60 minutes. In a second experiment pregnant guinea pigs (9/group) were given a dose of 0 or 60 mg/kg subcutaneously once at the end of pregnancy. The dams were sacrificed at intervals of 0.25-56 hours after the nitrite injection. Of the fetuses 96% died at 3 or more hours after nitrite administration. A relative narrow range was found between doses of sodium nitrite which had no effect on reproduction, doses that killed the fetuses and doses that killed the dams (Sinha and Sleight, 1971).

In another study (12/group) sodium nitrite was administered via the drinking water in concentrations of 0, 2000 or 3000 mg/l to pregnant rats. Non-pregnant females were similarly treated. The doses used corresponded to about 0, 200 or 300 mg/kg b.w.. Pregnant rats developed anaemia and had higher methaemoglobin levels than non-pregnant rats of the same treatment group. In the newborn rats of the treatment groups a considerably increased mortality was observed, especially in the 3 weeks before weaning. The mortality of the offspring was 6, 30 and 53%, respectively. In the offspring of the treated animals a significant growth reduction was noticed, while the birth weights were similar.

Pregnant rats were acutely treated with doses of sodium nitrite varying from 2.5-50 mg/kg b.w. show transplacental passage of the chemical with the production of methaemoglobin in the fetuses (Shuval and Grüner, 1972).

In a 2-generation reproduction study groups of rats were fed from the time of conception a diet containing 0, 240 or 460 mg/kg for 28 months. These doses correspond to about 0, 12 or 23 mg/kg b.w.. No effects were observed on the litter size, postnatal mortality, growth rate or life span (Shank and Newberne, 1976).

Teratogenicity

(See Table IV.)

In a teratogenicity/reproduction study 40 day old parent rats were fed heat-sterilized meat containing 0, 200, 1000 and 4000 mg sodium nitrite from day 40 onwards. These doses corresponded to about 0, 10, 50 or

200 mg/kg b.w.. The Flb generation was killed at day 21 of pregnancy. Fertility index, the number of preimplantation losses and the resorptions were not affected by nitrite treatment. In comparison with the control no differences in litter size, sex ratio and averaged weight of the pups were observed. No significant increase in the number of malformations was noticed (Carstensen and Hasselager, 1972, abstract only).

In an experiment with 2 groups of 10 and 15 pregnant rats sodium nitrite was administered on day 9 and 10 of pregnancy via the diet in concentrations of 3 and 10 g/kg diet. These doses corresponded to about 150 and 500 mg/kg b.w.. It was concluded that no embryotoxic and teratogenic effects were induced (Alexandrov and Jänisch, 1971).

Mutagenicity

In vitro exposure of purified DNA to nitrous acid leads to mutagenic activity. This was measured by the formation of lethal mutations in nitrous acid-treated DNA transformed to Bacillus subtilis (Strack et al., 1964; Bresler et al., 1968). Mutagenic activity of nitrite (or nitrous acid) has been reported in bacterial systems such as Escherichia coli and Salmonella typhimurium (Kaudewitz, 1959; Verly et al., 1967), in micro-organisms like fungi (various Aspergillus species, Neurospora crassa: WHO, 1978; De Serres et al., 1967), yeast (Saccharomyces cerevisiae) and in tobacco mosaic virus and bacteriophage T4 (Strack et al., 1964; WHO, 1978). In a study with mouse cells, sodium nitrite without metabolic activation did not lead to an increase in single strand breaks, but a dose-related increase in gene mutations and chromosome aberrations was found at relatively high doses. The mutagenic activity according to the authors was probably based on deamination of DNA and not on nitrosamine formation, since nitrosodimethylamine without metabolic activation hardly changed the mutation frequency (Kodama et al., 1976). Sodium nitrite administered in an acid environment (pH $\pm$ 5), induced an increase in 6-TG mutants in V79 hamster cells in vitro (Budayova, 1985). Chromosome aberrations were significantly increased in cultured hamster cells (Tsuda et al., 1976). Endoreduplication has also been reported (Tsuda and Kato, 1977). Sodium nitrite induced a sharp increase in "aberrant cells" obtained from human embryonic lung tissue (Stanford Research Institute, 1972, report not available).

After oral administration of sodium nitrite to 11 and 12 days pregnant Syrian hamsters, in cells cultured from the hamster embryos an increase was

found in drug-resistant mutations (8-AG and ouabain). Also a dose-dependent increase in micronucleus formation was found, although no enhanced number of chromosome aberrations was detected (Inui et al., 1979). It is possible that nitrite might not only act on nucleic acids, but also on proteins or -SH compounds, so that the mitotic apparatus, i.e. spindle-fiber formation, was also affected and damaged. This could be an explanation for the large number of cells with micronuclei in contrast with the lack of chromosome abnormalities.

In vitro morphological transformation of hamster cells by sodium nitrite was reported (Tsuda et al., 1973). Also in embryonic cells transformation was found in vitro whereas implantation of the transformed cells in vivo led to neoplasms (Inui et al., 1979).

Mutagenic activity was not found in two in vivo tests, a host-mediated assay and a micronucleus test with mice (Couch and Friedman, 1975; Hayashi et al., 1981). Administration of about 210 mg sodium nitrite/kg b.w. in drinking water to nonpregnant or 5-18 days pregnant rats, however, induced chromosome aberrations in bone marrow of both non-pregnant and pregnant animals as well as in the embryonic liver. The ratio of the number of metaphases with aberrations in treated and control animals, was higher for embryonic liver in comparison to adult bone marrow. This higher incidence may have resulted from higher numbers of cells in mitosis by shorter cell cycle times in embryonic tissues (El Nahas and Globus, 1984).

In another study no significant effects were found on metaphase chromosomes of bone marrow of adult rats. Experimental data such as dose levels were not known as the report was not available (Stanford Research Institute, 1972).

According to Zimmermann (1977) nitrite may exhibit mutagenic activity by three mechanisms. Firstly, nitrite may deaminate DNA-bases in single strand viral DNA. Spontaneous deaminations, however, are frequent and DNA-repair systems correcting these lesions are present in bacteria and probably mammalian cells as well. Secondly, formation of intra- or interstrand crosslinks between purine residues may occur, resulting in distortion of the helix in the case of double-stranded DNA. An induction of this type of lesions may be enhanced by the presence of molecules proximate to DNA, like polyamines, glycols, alcohols and phenols (Thomas et al., 1979). In the third place, nitrite may react with nitrosatable agents to N-nitroso compounds and thus indirectly exhibit mutagenic (and carcinogenic) activity

(NAS, 1981). See also 1.3.

1.2.3 Summary

Nitrate

The oral LD50 for mice varied from 2480 to 6250 mg sodium nitrate/kg b.w., for rats from 4860 to 9000 mg sodium nitrate/kg b.w., for rabbits the LD50 was 1600 mg and for cows 450 mg sodium nitrate/kg b.w.. From this data it can be concluded that cows are the most sensitive to nitrate. It is known from kinetic and metabolism studies that the toxicity of nitrate is largely determined by its conversion to nitrite and the subsequent formation of methaemoglobin. These findings are in agreement with the lower LD50 value in ruminants in which the conversion of nitrate to nitrite is considerably higher compared to non-ruminating calves and rodents. These observations are also supported by the finding that administration of the total dose in smaller amounts over 24 hours to cows, considerably reduced the toxicity (450 versus > 970 mg sodium nitrate/kg b.w.). Also a rather high subacute toxicity (200 mg/kg b.w. still had toxic effects) was found by Nighat et al. (1981) in rabbits, where nitrate was administered daily as a pulse dose in gelatin capsules.

To establish a no-toxic effect level the long-term study of Lehman (1958) with rats is considered to be the most appropriate; the results were confirmed by Til et al. (1985a and b). The no-toxic effect level for rats is then considered to be 500 mg sodium nitrate/kg b.w..

Part of the carcinogenicity studies are considered to be inadequate concerning duration of the experiment, number of animals used, route of administration, lack of information about the presence of N-nitroso compound content in the diet or the presence of N-nitroso compounds in the diet. Nevertheless, both from the only adequate study of Maekawa et al. (1982), and from the studies which were not quite up to the accepted standards, the conclusion was drawn that nitrate had no carcinogenic activity in experimental animals.

Nitrate is not mutagenic in bacteria and mammalian cells in vitro. In vivo experiments chromosome aberrations were induced in rat bone marrow, but it cannot be excluded that formation of N-nitroso compounds was responsible for the chromosome damage.

A no-toxic effect level of 10000 mg potassium nitrate/l drinking water corresponding to 1000 mg/kg b.w. was established from reproduction studies in guinea pigs.

In conclusion, nitrate is not carcinogenic and probably not mutagenic.

No adequate data concerning a possible teratogenic effect of nitrate are available.

Another piece of critical information restricting the value of the results of rodent - and especially rat - studies is the limited suitability of these animal species to study the toxicity of nitrate. As stated earlier, based on both toxicity and kinetic studies, the toxicity of nitrate depends largely on its conversion to nitrite. In the rat this conversion differs from that in man in whom this conversion is 4-7%. Therefore the no toxic effect level for nitrate probably has to be calculated from the no-toxic effect level of nitrite and the conversion rates known for man.

Nitrite

The acute oral LD50 values for mice varied from 175 to 220 mg sodium nitrite and the LD50 value for rats was 85 mg/kg b.w.. When a single dose of nitrite was divided into a number of smaller doses the toxicity of nitrite decreased.

From the chronic toxicity study with rats (Shuval and Gruner, 1972) a no-toxic effect level in rats was established at 100 mg sodium nitrite/l or 10 mg sodium nitrite/kg b.w.. The next higher dose level in this study, however, was 10 times higher.

Many studies dealing with the possible carcinogenicity of nitrite in mice and rats fed diets or drinking water containing nitrite were also inadequate, either with concern to duration, number of animals, route of administration, presence of N-nitroso compounds in the diet, lack of information on N-nitroso compounds content of the diet or because of the fact that the only nitrite dose group was intended to be a control group in studies designed to investigate the carcinogenic effects of simultaneous administration of nitrite and a nitrosatable amine.

The original conclusion from a large scale carcinogenicity study with rats fed a diet or drinking water containing nitrite and carried out by the FDA (Newberne, 1978; 1979) that there was an increased incidence of lymphomas, was reassessed by an Interagency Working Group of the U.S. Government. This group came to the conclusion that there was no increase in tumour incidence

of neither lymphomas nor other types (FDA, 1980a; b).

Although a few studies with nitrite revealed some slight increases in tumour incidences, which possibly resulted from the nitrosamines present in the diet fed to mice or rats, the final conclusion is that nitrite itself is not carcinogenic.

Studies on the carcinogenic effects of a combined administration of nitrite and amines are summarized under 1.3.

Nitrite exhibits fetotoxic effects. Fetotoxicity experiments in rats with nitrite administered via drinking water revealed effects at both levels tested (200 and 300 mg/kg b.w.), whereas in fetotoxicity and teratogenicity studies in rats with nitrite administered in feed a dose of 200 mg sodium nitrite per kg b.w. caused no effects.

Nitrite is mutagenic and causes morphological transformation in in vitro systems. Mutagenic activity was also found in a combined in vivo-in vitro experiment with Syrian hamsters. In vivo experiments led to conflicting results.

The mechanism in some in vitro systems probably is due to deamination, while in the in vivo experiments endogenous formation of N-nitroso compounds is likely to play a role.

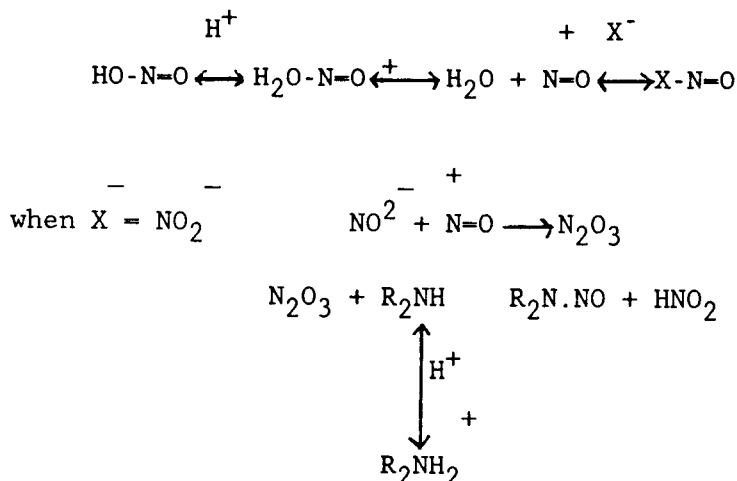
1.3 Nitrosation

1.3.1 Chemistry of formation of N-nitroso compounds

Nitrite can give rise to the formation of nitroso compounds by reaction with a nitrosatable compound.

Many different amino compounds including secondary and tertiary amines, secondary and tertiary amides, N-substituted ureas, guanidines and urethanes, can act as nitrosatable compounds. In the case of amines, amides and ureas the formed nitroso compounds are N-nitrosamines, N-nitrosamides and N-nitrosoureas. The most common nitroso compounds are those derived from secondary amines. The following equilibria are involved:

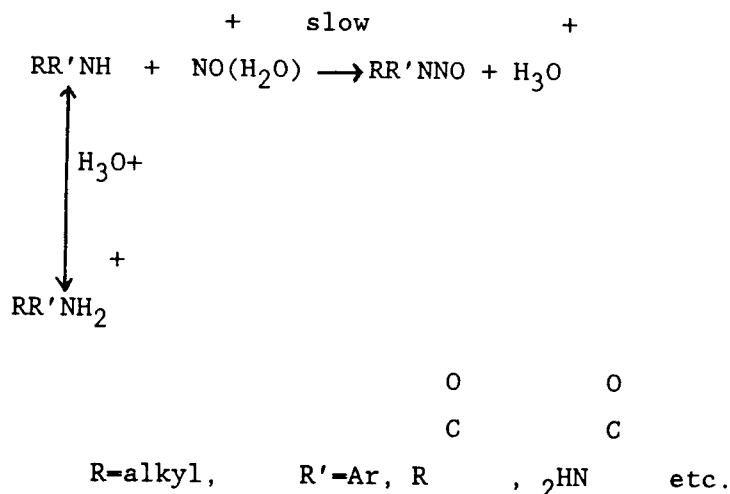
scheme 1



The rate of formation is pH dependent and proportional to the concentration of the unprotonated amine (= inversely related to the basicity of the amine) and the concentration of N₂O₃ and hence to the square of the NO₂⁻ concentration. While the N₂O₃ concentration increases with decreasing pH, the R₂NH concentration decreases and a pH optimum in the range of 2.5-3.3 is commonly observed (Mirvish, 1975; Challis, 1981,1985; Foster et al., 1981).

Many nitrosatable compounds (e.g. some aromatic amines, amides, ureas) are too unreactive to combine readily with N₂O₃. They react by another pathway, attributed to a direct reaction of the neutral substrate with either H₂ONO⁺ or NO⁺. Usually these reactions are quite slow at pH >3, but they become progressively faster with increasing acidity (Challis, 1981).

scheme2



Nitrosation occurs especially rapidly with secondary amines of weak basicity (e.g. morpholine, piperazine, N-methylaniline), N-alkylureas, N-alkylcarbamates and aminopyrine. Nitrosation occurs relatively slowly with strongly basic amines, such as dimethylamine, and simple N-alkylamides. Nitrosation of tertiary amines, yielding dialkylnitrosamines, and nitrosation of guanidines, yielding nitrosocyanamides and nitrosoureas, occurs relatively slowly (Mirvish, 1975).

Catalysis of N-nitrosamine formation by nucleophilic anions at pH 2-5 has been widely observed (in scheme 1, X⁻ is a nucleophilic anion). The catalytic order is SCN⁻ > I⁻ >> Br⁻ > Cl⁻ >>> phosphate or carboxylate. Acceleration by SCN⁻ and I⁻ have attracted much attention because of their in vivo relevance. Salivary SCN⁻ levels have been enhanced in smokers (3-4x) and I⁻ is present in gastric secretion. Nitrosation of amides and related compounds is not catalysed by nucleophilic anions. It is not known whether guanidines behave like weakly basic amines (strong anion catalysis) or like amides (no catalysis).

Effective inhibition of nitrosation requires materials which react readily with and convert the nitrosating agent to innocuous products e.g. compounds which either reduce HNO₂ to N₂ or NO or bind the NO⁺ group irreversibly.

Sulphur dioxide and bisulphite ion, ascorbic acid, tocopherols, gallic acid, thiols, several dihydroxy phenols and some other well-established synthetic and natural antioxidants are inhibitors of nitrosation (Challis,

1981).

1.3.2 Occurrence of nitrosation

Nitrosation can occur in a.o. food, cosmetics and tobacco products during storage and processing.

In 1957, fur farms lost many of their animals because of a malignant liver disease and herring meal was suspected to be the toxic agent. Later studies showed that amines, normally present in fish, can react with sodium nitrite, used as a preservative, to produce dimethylnitrosamine which can cause liver malignancy (as cited in Koppang, 1980).

Since then, N-nitroso compounds have been detected in many products (see Table VII).

Endogenous nitrosation can occur in the stomach by reaction of nitrite, formed by reduction of nitrate in the oral cavity or in the stomach itself, or by reaction of nitrite ingested via food, with a nitrosatable compound. The endogenous nitrosation of dietary precursors has been studied;

- a) in vitro; incubation of precursors of nitroso compounds with gastric juice (or under simulated gastric conditions).
- b) in vivo; examination of stomach contents or saliva at intervals after administration of precursors of nitroso compounds.
- c) in vivo; determination of nitroso compounds in blood, urine, faeces or intestinal contents after administration of precursors.
- d) 1. toxicity, 2. mutagenicity and 3. carcinogenicity studies in animals with nitrate or nitrite and a nitrosatable compound.

a) In vitro gastric simulation studies

In vitro studies with animal or human stomach contents or gastric juice together with nitrite and a known nitrosatable compound were performed.

Alam et al. (1971a) demonstrated the formation of nitrosopiperidine after incubation of gastric juice of rats with nitrite and piperidine.

Rickard and Dorrough (1984) incubated stomach contents of rats and guinea pigs with carbaryl, carbofuran or methylurea and sodium nitrite. Less than 1% nitrosation of the amides occurred in stomach contents of rats (pH 4-5) while in stomach contents of guinea pigs (pH 1.2-2.6) 19-37% nitrosation was found.

Sen et al. (1969) incubated gastric juice of rats, rabbits, cats, dogs and men with diethylamine and nitrite. More diethylnitrosamine was found in

gastric juice of rabbits and man (pH 1-2) than in gastric juice of rat and dog (pH 4-5 and 7.4, respectively).

Sander (1967), Walters et al. (1979), Ziebarth and Teichmann (1980) and Kubachi and Kupryzewski (1980) demonstrated the formation of nitroso compounds after incubation of human gastric juice with nitrite and several nitrosatable compounds (dimethylamine, diethylamine, L-proline, carbaryl, and a number of drugs)

Kyrtopoulos et al. (1985) showed the formation of nitroso compounds after incubation of fasting human gastric juice with different amounts of nitrite at pH 2-7.

After incubation of gastric juice of rats with nitrate and the cyclic amine, piperidine, nitrosopiperidine was detected (Alam et al., 1971b).

Human saliva was incubated without or with nitrate at pH < 2. Nitroso compounds (nitrosamides and nitrosamines) were determined in the incubation mixtures. In 11/100 samples after incubation without nitrate and in 14/100 samples after incubation with nitrate positive results were found (100-500 µg/l as N-nitrosopyrrolidine). Before incubation 7/100 samples showed positive results (Hart and Walters, 1983).

In a few studies food products were incubated with (artificial) human gastric juice and nitrosamines were determined in the mixture.

Walters et al. (1974) treated homogenates of milk and cheese at pH 1.0 and 3.0 with nitrite levels, similar to human gastric conditions. Volatile nitroso compounds were detected only in the cheese homogenate while non-volatile nitroso compounds were detected both in milk and cheese homogenate.

In a later study (Walters et al., 1979), slurries of meals (including fried eggs, bread, butter, cheese, biscuits, milk and luncheon meat) were incubated with human gastric juice and nitrite at pH 2.0. A mean value of 6.7 mg N-nitrosopiperidine/kg of food was found after 15 min. Prolonging of the incubation time did not cause a further rise.

Groenen et al. (1982) incubated a great number of food products with artificial human saliva and artificial human gastric juice and determined volatile nitrosamines. Incubation mixtures with fish samples contained < 1 upto 44 µg dimethylnitrosamine per portion (2.5-250 g). Low levels were found in smoked sausage (0.1 µg/250 g) and in cinnamon (0.2 µg/0.25 g). The majority of the food products did not form volatile nitrosamines.

Walters et al. (1979) nitrosated a tobacco smoke condensate under

exhaustive (high NO_2^- content) and simulated gastric conditions. 880 mg extractable nitroso compounds (as N-nitrosopyrrolidine)/kg condensate were found after exhaustive nitrosation compared with 1.2 mg/kg before nitrosation. Under simulated gastric conditions 12 mg extractable nitroso compounds (as N-nitrosopyrrolidine)/kg condensate were detected.

b) In vivo nitrosation, detection in stomach contents or saliva

In several animal studies nitroso compounds have been detected in the stomach contents after administration of nitrite and a known nitrosatable compound. The following amino compounds were nitrosated in the stomach of animals in the presence of nitrite: dimethylamine, diethylamine, piperidine, pyrrolidine, piperazine, diphenylamine, methylbenzylamine, methylaniline, methylurea, ethylurea, dimethylglycine, phenmetrazine, carbaryl, carbofuran, trimorphamide, ziram, thiram, Alar (Lijinsky, 1981; Eisenbrand et al., 1974; Walker, 1981; Iqbal et al., 1980; Borzsonyi et al., 1980; Sen et al., 1974; Rickard and Dorough, 1985).

In a few studies nitrate and a known nitrosatable compound were administered.

Alam et al. (1971b) demonstrated in vivo formation of nitrosopiperidine in the stomach of rats from nitrate and piperidine but to a lesser degree than from nitrite and piperidine at comparable doses.

Greenblatt et al. (1972) did not detect any nitrosophenmetrazine in the stomach of rats after oral administration of nitrate and phenmetrazine.

Also in man the formation of nitroso compounds has been investigated. Sander and Seif (1969) detected diphenylnitrosamine in the stomach of 11/31 gastric patients after intake of sodiumbicarbonate, nitrate and diphenylamine. Bellander et al. (1984) detected mononitrosopiperazine in gastric juice 30 min. after oral administration of piperazine to 4 fasting male volunteers.

Groenen et al. (1984) administered to volunteers meals with different nitrate contents viz. fish with high-nitrate vegetables, fish with low-nitrate vegetables and meat/or eggs with high-nitrate vegetables. 0.5-2 Hours after consumption of the meals, dimethylnitrosamine levels upto 7.6, 3.7 and 0.9 $\mu\text{g/kg}$ gastric liquid, respectively were found. In some cases peaks of 16-30 $\mu\text{g/kg}$ were found 4-5 hours after consumption of a meal with fish and high-nitrate vegetables. A great interindividual variation in nitrosamine formation was observed. In some cases diethylnitrosamine was

detected additionally at concentrations upto 13 µg/kg 0.5-3 hours after consumption of a meal with fish and high-nitrate vegetables. Dipropyl- and dibutyl nitrosamine, N-nitrosopiperidine, N-nitrosomorpholine and N-nitrosopyrrolidine were not detected. In fasting gastric juice < 0.2-0.7 µg dimethylnitrosamine/kg was found.

Walters et al. (1979) administered homogenates of meals consisting of egg, milk and luncheon meat per oral tube to a volunteer. The stomach contents were analysed for volatile nitrosamines upto 60 min. after ingestion of the homogenates. Trace amounts of N-nitrosopiperidine (0.36 µg/kg) were found occasionally after 30 min. N-Nitrosopyrrolidine and volatile nitrosamines of the simple dialkyl type were not detected.

Analysis of samples of gastric juice from healthy individuals as well as from patients with morphological changes of the gastric mucosa and from patients who had undergone gastric surgery, did not show any indication for contents of volatile nitrosamines, although some samples contained nitrite (Eisenbrand et al., 1981).

Gastric contents of volunteers, receiving meals with fish or beef or bacon together with spinach and vegetable juice, were examined for nitrosamines. No significant increases in nitrosamine content were observed (Lakritz et al., 1982).

While Bartsch et al. (1984) did not detect a higher nitrosation in fasting gastric juice of patients with chronic atrophic gastritis and similarly found no increase in total gastric nitroso compounds in duodenal ulcer patients after cimetidine treatment, Reed et al. (1984) found a significantly higher amount of nitrate-reducing bacteria, nitrite and nitroso compounds in fasting gastric juice of patients with partial gastrectomy than in normal controls.

10 Volunteers on 10 separate days within 10 weeks received 16 or 64 mg NaNO₃ in 250 ml of water (about equal or 4x the recommended WHO limit of 50 mg NO₃⁻/l). Nitroso compounds (amines and amides) in saliva before and 1 hour after ingestion of nitrate were determined. The nitrate ingestion did not affect the level of nitroso compounds in saliva (Hart and Walters, 1983).

Eisenbrand et al. (1981) did not detect any dimethylnitrosamine (< 0.1 µg/l) in saliva of 27 volunteers just after breakfast or lunch.

c) In vivo nitrosation, detection in blood, urine, feces or intestinal contents

It has to be noted that artefact free analysis of biological samples containing $< 1 \mu\text{g/kg}$ of nitrosamines, is difficult. Therefore the early reports (upto and including 1980) of volatile nitrosamines in human blood, urine and feces may be incorrect (Eisenbrand et al., 1981; Lee et al., 1981; Fine et al., 1982; Wagner and Tannenbaum, 1985).

Blood

In several studies blood of normal human subjects showed dimethylnitrosamine levels ranging from $0.1\text{-}2.5 \mu\text{g/l}$ (Lakritz et al., 1980, 1982; Yamamoto et al., 1980; Gough et al., 1983) and diethylnitrosamine levels from $<0.1\text{-}0.4 \mu\text{g/l}$ (Melikian et al., 1981; Gough et al., 1983).

Fine et al. (1977) and Gough et al. (1983) found an increase in blood dimethylnitrosamine levels of $0.3\text{-}0.4 \mu\text{g/l}$ in 1 and 2 human subjects, respectively after consumption of a meal with bacon, spinach, bread and beer. However Melikian et al. (1981), administering the same meal as Fine et al. (1977) without beer, found an increase in blood dimethylnitrosamine levels in 2 subjects and a decrease in another subject.

Kowalski et al. (1980), Yamamoto et al. (1980), Lakritz et al. (1982) and Groenen et al. (1984) observed only very slight or no increases in blood nitrosamine levels of human subjects after consumption of nitrate/nitrite and/or amine rich meals.

Ellen et al. (1982) did not detect dimethyl- or diethylnitrosamine in blood of 23 patients ingesting $2.5\text{-}9 \text{ g}$ of ammonium nitrate daily.

Urine

Urine of healthy persons contained only traces of nitrosamines or no nitrosamines at all. In persons with urinary tract infections nitrosamines may be present in urine (Hicks et al., 1977; El-Merzabani et al., 1979; Kakizoe et al., 1979; Eisenbrand et al., 1981).

Lakritz et al. (1982) did not find an increase in urinary nitrosamine levels of human subjects after consumption of meals with fish or beef (source of amines) or bacon (source of preformed nitrosamines) in combination with spinach and vegetable juice (source of nitrate/nitrite).

Ellen et al. (1982a) did not detect volatile nitrosamines in urine of 23 patients, even after daily oral ingestion of large amounts of ammonium

nitrate (2.5-9 g), for prevention of development of renal stones.

After ingestion of piperazine by human volunteers, Bellander et al. (1984) detected mononitrosopiperazine in the urine. Spiegelhalder and Preussmann (1984) found dimethylnitrosamine in urine of human volunteers after ingestion of aminopyrine together with alcohol containing beverages and/or high-nitrate vegetables.

Ohshima and Bartsch (1981) developed a quantitative method for measuring nitrosation in man. Urinary levels of nitrosoproline, a non-carcinogenic and non-mutagenic nitrosamine, were measured after ingestion of nitrate and proline. Because nitrosoproline is hardly metabolized, urinary excretion could be used as a quantitative indicator of nitrosation in vivo. The amount of total nitrosoproline excreted in urine, was found to be proportional to the proline dose and increased exponentially with the nitrate dose. With the highest dose of nitrate (325 mg) together with the highest dose of proline (500 mg) 16.6 to 30.0 (mean 23.3) μg nitrosoproline was formed in 24 h. Intake of 260 mg nitrate together with 500 mg proline resulted in the formation of ca. 10 μg nitrosoproline. At intakes of 195 or 130 mg nitrate together with 500 mg proline ca. 3 and 2 μg nitrosoproline, respectively, was formed. At the intake of 65 mg nitrate together with 500 mg proline the amount of nitrosoproline formed was hardly detectable. When different amounts of proline were given (60-500 mg) together with 325 mg nitrate 2.7-30 μg nitrosoproline was formed.

In patients with chronic atrophic gastritis, Bartsch et al. (1984) did not find a higher amount of urinary nitrosoproline or other nitroso amino acids after ingestion of nitrate and proline, than in a control experiment with healthy persons. Similarly, cimetidine treatment of 17 duodenal ulcer patients did not lead to an increase in urinary nitrosoproline levels.

Faeces

Wang et al. (1978) detected dimethyl- and diethylnitrosamine in normal human feces at levels upto 1.5 and 13 $\mu\text{g}/\text{kg}$, respectively; lower levels (ca. 1 $\mu\text{g}/\text{kg}$) of dibutylnitrosamine, nitrosopyrrolidine and nitrosomorpholine were found, too. However, in later studies (Archer et al., 1981; Eisenbrand et al., 1981; Lee et al., 1981) no volatile nitrosamines in faeces of healthy persons or patients could be detected. Suzuki and Mitsuoka (1981) found nitrosamines in faeces of Japanese individuals and reported an increase in the nitrosamine levels after

consumption of a Western diet. In a later study of the same authors (1985), the positive results were ascribed to artefactual generation of nitrosamines during analytical procedures.

Intestinal contents

Alam et al. (1971a; 1971b) demonstrated in vivo formation of nitrosopiperidine from piperidine and nitrite or nitrate in the intestinal contents of rats.

Suzuki and Mitsuoka (1984) and several authors in earlier literature (as cited in Suzuki and Mitsuoka, 1984) reported nitrosamine formation by intestinal bacteria. Suzuki and Mitsuoka (1984) showed that nitrosamine formation, from nitrite and a secondary amine, by some intestinal bacteria, was due to enzymatic catalysis.

In contrast, Mallett et al. (1985) found no differences in urinary nitrosoproline excretion by conventional microflora and germ-free rats after ingestion of nitrate and proline. This suggests no involvement of intestinal microflora in nitrosation of proline. Recently it was found that intestinal bacteria do not catalyse nitrosation of proline at pH > 6. This is a serious draw-back of the NPRO-test, as nitrosation of other secondary amines is catalysed by intestinal bacteria at elevated pH-levels (Crespi et al., 1986).

d) 1. Toxicity, 2. mutagenicity and 3. carcinogenicity studies in animals with nitrate or nitrite and a nitrosatable compound

Toxicity studies

After the oral ingestion of high doses of sodium nitrite together with a secondary amine (dimethylamine, methylbenzylamine) or the tertiary amine aminopyrine, toxic effects (progressive inertia, anorexia, ascites, weight loss, mortality, hepatic necrosis) characteristic for nitrosamines, were observed in mice or rats. Administration of sodium nitrite or the amine alone, did not reveal the above reported effects (Asahina et al., 1971; Astill and Mulligan, 1971; Lyinski and Greenblatt, 1972).

Montesano and Magee (1971) demonstrated the methylation of nucleic acids in stomach, liver and small intestines of rats after the combined ingestion of ¹⁴C-labelled methylurea and sodium nitrite. No methylation was observed after the ingestion of ¹⁴C-labelled methylurea alone.

Mutagenicity studies

Nitrosation products of several drugs (by treatment with NaNO_2 in acid medium) were shown to possess mutagenic activity in a bacterial assay with Salmonella typhimurium (Andrews et al., 1980).

Treatment of some food products (fish, beans, borscht) with sodium nitrite (1000 and 5000 mg/kg) at pH 3.0 led to the development of mutagenic activity in S. typhimurium in the absence and the presence of a metabolic activation system (Marquardt et al., 1977; Weisburger et al., 1980). Also Tomita et al. (1984) detected mutagenic activity of nitrosation products of Japanese foodstuffs (after treatment with nitrite at pH 4.2) in S. typhimurium without and with metabolic activation.

Inui et al. (1978; 1980) administered orally sodium nitrite and morpholine or amidopyrine to pregnant Syrian hamsters. Gene mutations were found in cultured embryonic cells, most probably due to transplacental activity of the nitrosamine formed in the mother.

In intra-sanguineous host-mediated assays with mice, combined administration of nitrite and dimethylamine, morpholine or aminopyrine, induced mutagenic activity in the test organisms S. typhimurium, S. typhimurium and Schizosaccharomyces pombe, respectively (Barale et al., 1981; Edwards et al., 1979; Whong et al., 1979).

Brambilla et al. (1985) showed that nitrosation products of several drugs (after treatment with nitrite in acidic medium) caused DNA fragmentation in Chinese hamster ovary cells in vitro.

Unscheduled DNA synthesis (UDS) was determined in leucocytes of 10 human subjects after the consumption of meals containing varying amounts of nitrate, nitrite or nitrosamines. In 6/10 subjects UDS was increased significantly but no correlation with dietary nitrate, nitrite and nitrosamine levels or with blood nitrosamine levels could be detected (Kowalski et al., 1980). Also Miller (1980) did not observe any effect of ingested nitrate/nitrite (lettuce) on UDS in leucocytes of human subjects after consumption of an amine containing meal (fish).

Carcinogenicity studies

Carcinogenicity studies in animals, receiving orally a nitrosatable compound and nitrite, demonstrated the induction of tumours characteristic for the presumed corresponding nitroso compound.

The amines and amides, causing tumour induction in these studies, included

amidopyrine, heptamethyleneimine, oxytetracycline, morpholine, N-methylbenzylamine, N-methylaniline, N-methylcyclohexylamine, imidazolidinone, methylurea, ethylurea, N,N-dimethylurea, N-methyl-N'-nitroguanidine, piperazine, N6-methyladenosine, and disulfiram. Negative results were found for dimethyl- and diethylamine in mice and rats, respectively.

In model experiments of this type high doses of nitrite and amines or amides were used. These conditions are usually quite unrealistic by comparison with human exposures under normal conditions (Lijinsky, 1981; Preussmann and Stewart, 1984).

Two dose-response studies in mice and rats, respectively are available.

Shank and Newberne (1976) found an increased incidence of liver cell carcinomas and angiosarcomas in the liver and the lung in rats at a sodium nitrite concentration of 1000 mg/l drinking water and only 5 mg morpholine per kg feed. Lower nitrite levels (50 and 5 mg/l) in combination with 5-1000 mg morpholine per kg feed, revealed the same tumour incidences as in a control group with 1000 mg morpholine per kg feed.

Greenblatt and Mirvish (1972) found a significantly increased incidence of lung adenomas in mice, given orally 1000 mg sodium nitrite per l. drinking water in combination with 700 mg piperazine/kg feed. Lower nitrite levels (250 and 500 mg/l) in combination with 6250 mg piperazine/kg feed, also caused a significant tumour induction. The lowest nitrite level (50 mg/l) in combination with 6250 mg piperazine/kg of feed revealed tumour incidences comparable to controls.

Weisburger et al. (1980) treated homogenates of mackerel fish with sodium nitrite at pH 3.0. The extracts were given per stomach tube 3x/week for 6 months to Wistar rats. 12-18 Months after feeding, adenomas and adenocarcinomas in glandular stomach, squamous cell carcinomas in forestomach and adenocarcinomas in the small intestine and pancreas were observed.

1.3.3 Summary

Nitrosation by reaction of nitrite with a nitrosatable compound has been found to be a pH dependent process. Nitrosation can occur in a.o. food, cosmetics and tobacco products during storage or processing.

Endogenous nitrosation has been studied in vitro and in vivo. In vitro gastric simulation studies demonstrated the nitrosation of several amino

and amido compounds and of some constituents of food products (especially fish).

In vivo studies in animals clearly demonstrated the occurrence of nitrosation.

In some qualitative studies in man endogenous nitrosation has been demonstrated. Among other traces of nitrosamines (dimethylnitrosamine and diethylnitrosamine) in gastric fluid of man has been detected after simultaneous consumption of high-nitrate vegetables and fish.

A quantitative determination of endogenous nitrosation in man has been performed by Ohshima and Bartsch (1981). The urinary excretion of nitrosoproline after the oral intake of nitrate and proline had been measured. The amount of total nitrosoproline excreted in the urine was found to be proportional to the proline dose and increased exponentially with the nitrate dose. At the highest dose of nitrate (325 mg) and proline (500 mg) a mean of 23.3 µg nitrosoproline was formed. At intakes of 195 or 130 mg nitrate together with 500 mg nitrosoproline ca. 3 and 2 µg nitrosoproline have been formed. At the intake of 65 mg nitrate together with 500 mg proline the amount of nitrosoproline formed was hardly detectable. It has to be noted that dimethylamine and diethylamine are nitrosated less rapidly than proline, while morpholine and pyrrolidine are nitrosated more rapidly than proline.

1.4 Observations in man

1.4.1 Toxicity of nitrate and nitrite in man

Like in animals, the toxicity of nitrate in man depends on the conversion of nitrate to nitrite. For this reason babies and patients with hypo- or achlorhydria and/or stomach lesions are to be considered as special risk-groups (see 1.1). These patients might also be more susceptible to the toxicity of nitrate.

In the literature human lethal doses of 4-50 grams NO_3^- (corresponding to 80-833 mg NO_3^-/kg body weight) are described. Toxic doses - with methaemoglobin formation as a criterion for toxicity - were found between 2 and 5 grams (Corré and Breimer, 1979) and 6 and 9 grams of NO_3^- (Fassett, 1973). These values correspond to 33-83 and 100-150 mg NO_3^-/kg body weight, respectively. Fassett reported a rapidly occurring severe gastro-enteritis with abdominal pain, blood in the urine and faeces as symptoms of acute

nitrate intoxication. Repeated doses gave rise to dyspepsia, mental depression, headache and weakness.

The data partly originate from relatively old publications, some of them without details about age or gastric conditions. The low values of these lethal and toxic doses are difficult to interpret. Against these values pleads among others the absence of toxic symptoms in 12 volunteers receiving 9.5 grams of sodium nitrate in 1 hour intravenously, while in 2 of 12 other persons administered 7-10.5 grams of ammonium nitrate orally in one dose, vomiting and diarrhoea occurred (Ellen et al., 1982b). The lethal dose of nitrate for adults probably amounts to around 20 grams NO_3^- , corresponding to about 330 mg NO_3^-/kg b.w. (Leu et al., 1986; Ellen, 1986). In infants under the age of 3 months the conversion of nitrate to nitrite and methaemoglobin formation is high as discussed previously in 1.1. Gastrointestinal disturbances play a crucial role here; the reduction of nitrate to nitrite in the stomach is enhanced by bacterial growth at the high pH in the stomach of these infants. The toxic effects are therefore induced at a much lower dose level of nitrate than in adults. According to Corré and Breimer (1979) assuming a 80% reduction of nitrate to nitrite in these young infants, the toxic dose level was calculated to vary from 1.5-2.7 mg NO_3^-/kg b.w., using 10% formation of methaemoglobin as toxicity criterion (Winton, 1971). This value is in good agreement with the toxic dose of 8.6 mg NO_3^- calculated for babies by Grau. In the same report a lethal dose for babies of 43.2 mg NO_3^- is calculated based on haemoglobin/methaemoglobin transfer stoichiometry (WHO, 1985).

However, in reported cases of infant methaemoglobinaemia the amounts of nitrate ingested are higher: 37.1-108.6 mg/kg b.w., with an average of 56.7 mg/kg.

Acute intoxications have been reported due to drinking of well with a high nitrate content (WHO, 1978; NAS, 1978). Of all reported cases of infantile methaemoglobinemia 97.7% occurred in areas with a nitrate drinking water content of more than 44.3-88.6 mg NO_3^-/l (WHO, 1985). In The Netherlands, these intoxications have occurred sporadically in the last two decades. However, the evaluation of cases of infantile methaemoglobinemia in relation to nitrate intake is difficult because of often occurring simultaneous bacterial contamination of drinking water, and of bacterial infections in infants, which may influence the reduction of nitrate to nitrite as well as the endogenous synthesis of nitrate. Hegesh and Shiloah

(1982), for example, found a significantly increased nitrate blood content, in correlation with an increased methaemoglobin content, in infants with acute diarrhea, whereas the intake of nitrate and nitrite in these infants low (2-7 mg/day). See also 1.1.3.

In healthy babies of 11 days to 11 months of age oral treatment for several days with 50 or 100 mg NO_3^- /kg b.w., increased methaemoglobin levels (5.3-7.5%) but no cyanosis was seen. In 6-7 week old babies just restored from previous methaemoglobinemia after administration of 100 mg NO_3^- /kg b.w., cyanosis and increased methaemoglobin concentrations (up to 11%) were found. Details of individual babies about age and days of treatment were not given (Cornblath et al., 1948).

Nitrite is more toxic to young infants than to adults, due to the higher methaemoglobin formation in infants as was discussed in 1.1. Accidental human intoxications have been reported due to the presence of nitrite in food. From these sources it could be deduced that the oral lethal dose for man varied from 33-250 mg NO_2^- /kg b.w. (Corré and Breimer, 1979). The lower doses apply to children and elderly people. Toxic doses giving rise to induction of methaemoglobinaemia ranged from 1 to 8.3 mg/kg b.w. (Winton et al., 1971; Simon, 1970).

Another source of information with respect to nitrite toxicity in humans is the use of sodium nitrite as medication for vasodilation or as antidote in cyanide poisoning. Doses of 30-300 mg/person or 0.5-5 mg/kg b.w. did not cause any toxic effects (NAS, 1981).

Next to babies under 3 months of age several other categories of individuals with altered physiological states or with either hereditary or acquired disease may also be predisposed to the development of nitrite or nitrate induced methaemoglobinemia. These include pregnant women (Metcalf, 1961), individuals with glucose-6-phosphate dehydrogenase deficiency (Kohl, 1973), adults with reduced gastric acidity (including those being treated for peptic ulcer or individuals with chronic gastritis or pernicious anemia), and a rare group with a hereditary lack of NADH or methaemoglobin reductase activity in their red blood cells (Scott, 1960).

Individuals with hereditary structural abnormalities in haemoglobin, referred to as haemoglobin Ms, are probably also at increased risk from dietary nitrate or nitrite (Jaffé and Heller, 1964; cited in NAS, 1981).

1.4.2 Relation between nitrate and nitrite intake, the subsequent endogenous formation of N-nitroso-compounds and possible risk of (stomach) cancer in man

Several authors (Table V and VI) suggest that the risk for the development of stomach cancer is positively correlated with three factors: 1) the nitrate level of the drinking water, 2) the urinary excretion of nitrate and 3) the occurrence of atrophic gastritis.

During the last three decades the incidence of stomach cancer is decreasing. It has been suggested that this was caused by factors like the significant reduction of nitrate and nitrite concentrations in cured meat, and the increasing use of refrigerators and freezers (Hartmann, 1983).

The incidence of gastric cancer is still high in countries with a frequent consumption of salted fish (Japan, Iceland, Chile) or countries with long winters and consequently prolonged food preservation (Eastern Europe, USSR, China).

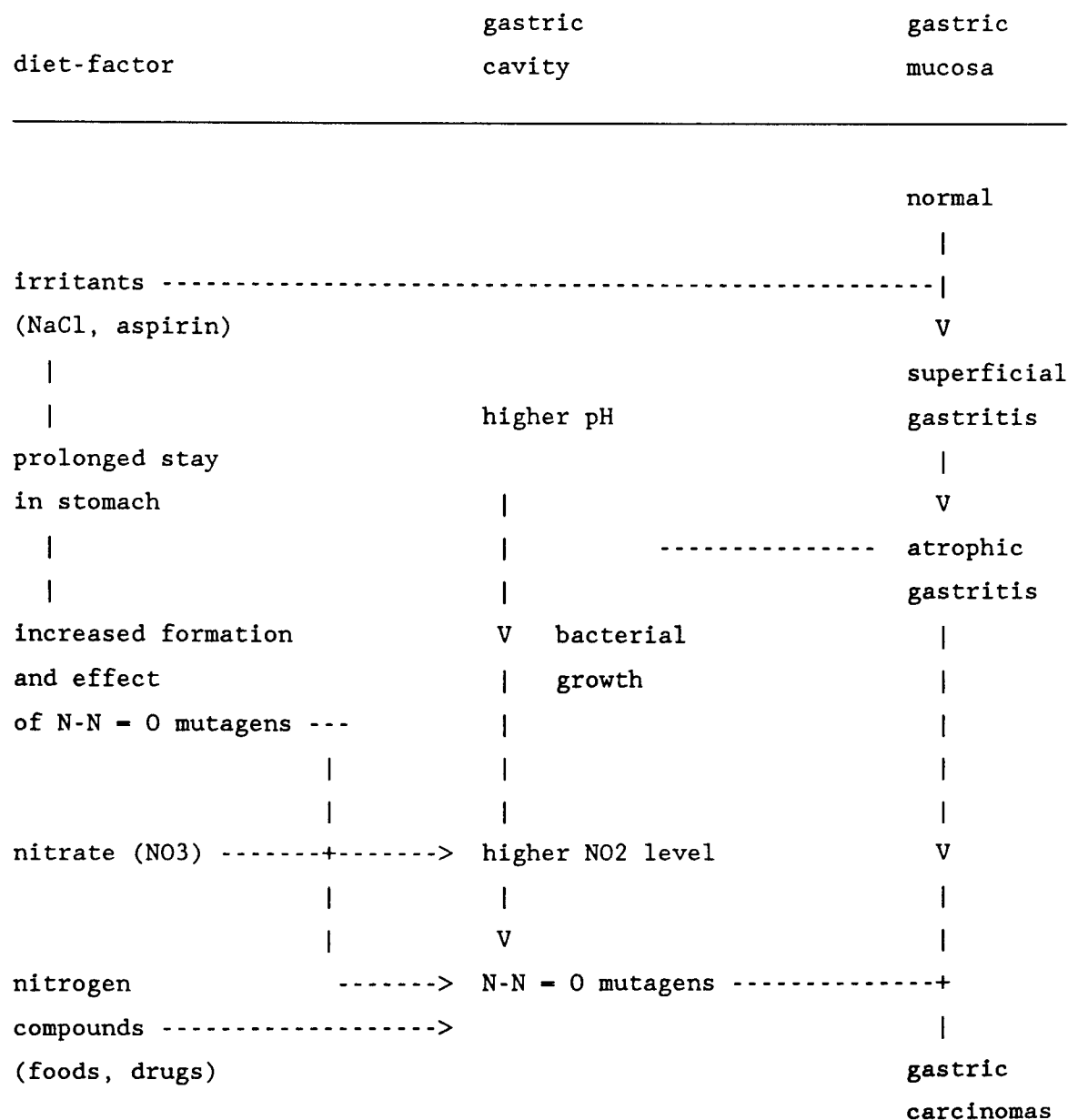
The daily nitrate intake seemed to be associated with the development of gastric cancer in a number of epidemiological and related studies (Weisburger and Raineri, 1975; Fraser et al., 1980; NAS, 1981). Also Fine et al. (1982) suggested an association between nitrate intake and gastric cancer mortality by combining previously published data on daily nitrate intake in different countries with gastric cancer mortality rates ($r = 0.88$).

Based on the available results of epidemiological and related research concerning the association of food components and the development of stomach cancer two hypotheses were drafted (Joossens and Geboers, 1981; Food Council Report, 1986): 1). The salt hypothesis in which a large intake of salt is considered as an important factor and 2) the nitrate/nitrite hypothesis as is dealt with in this chapter. As nitrate and other salts are present in the diet, a combination of both hypotheses is likely (Correa et al., 1975; 1983; Weisburger et al., 1981).

Several factors or conditions which can influence the formation of gastric tumours are outlined in the following figure. For the correlation between nitrate intake and tumour incidence these include factors influencing the reduction from nitrate to nitrite. These factors, discussed in detail in 1, involve the biotransformation of nitrate and include factors like thiocyanate (smokers versus non-smokers), iodide, age (increasing salivary

nitrate and nitrite levels with increasing age), conditions for bacterial growth in the gastro-intestinal tract (pH of the stomach or type of indigestible material in the diet), and antacida medication (Armijo et al., 1981a and b; Boyland and Walker, 1974; Eisenbrand et al., 1980; Forman et al., 1985; NAS, 1981; Reed et al., 1981b; Ruddell et al., 1978; Tannenbaum et al., 1979; Tannenbaum and Correa, 1985; Ward, 1984).

Model for the aetiology of gastric carcinomas (partly after Correa, 1983)



Concerning the correlation between nitrate or nitrite intake and gastric

tumour incidence factors modulating the formation of carcinogenic N-nitroso compounds are also important. These modulators of the nitrosation of amines and amides were discussed in 1.3 and include the role of thiocyanate, high salt intake, pH of the stomach, vitamin C or other dietary components, medication (cimetidine and other antacida), and gastric lesions or disorders (Armijo et al., 1981a, b; Forman et al., 1985; Mirvish, 1985; Risch et al., 1985; Tannenbaum and Correa, 1985). In many epidemiological and related studies concerning the nitrate or nitrite intake and the occurrence of gastric tumours, these factors are therefore discussed by several authors (Table V and VI).

The majority of the studies revealed no or in some cases a negative correlation between nitrate intake and gastric cancer. A high intake of certain vegetables, though an important source of nitrate, seems to be associated with a lower risk of gastric cancer. Protective factors such as ascorbic acid, simultaneously present in these products may be involved.

Epidemiological studies have been carried out in different countries concerning the relation of gastric cancer and nitrate exposure via drinking water. Salivary nitrite levels in volunteers were strongly increased after consumption of drinking water containing 200 mg NO_3^-/l in comparison to 50 mg NO_3^-/l (EC guideline). However, in studies on large populations from Chile, Denmark, England, France and Hungary no proof of a correlation between nitrate in drinking water and stomach cancer was found. This still held true when the analysis was restricted to urban areas with nitrate levels above the EC guideline (Hart and Walters, 1983; Hill et al., 1973; Zaldivar and Wetterstrand, 1978; Juhasz et al., 1980; Davies, 1980; Jensen et al., 1982; Vincent, 1983; Beresford, 1985; WHO, 1985). See also Table V. The originally reported positive association for females in the mining town Worksop (Hill et al., 1973) -after correction for mining area and social class- was not significant any more (Davies, 1980).

Also no association was found between nitrate concentration in food alone or in combination with drinking water in Chile and England, when populations from high- and low-risk areas for stomach cancer were compared (Armijo et al., 1981b; Forman et al., 1985).

Exposure by environmental pollution or food via natural fertilizers in Chile led to a significant association between nitrate load and gastric cancer (Armijo et al., 1975; Zaldivar, 1977). See also Table V.

Studies of gastric cancer mortality have been performed in occupationally

exposed fertilizer workers. No evidence of an association with gastric cancer has been found (Fraser et al., 1982; Al-Dabbagh et al., 1986). See also Table V.

A study on 556 grinders occupationally exposed during the years 1958 to 1976 to cutting fluids containing nitrite and amines, did not reveal an increased risk of cancer (Järvholm et al., 1986).

Individuals with an achlorhydric stomach, and persons on cimetidine and antacida medication, do present a special risk group. Chronic gastritis, especially the atrophic form seems to be an important intrinsic factor in the genesis of stomach cancer (Cuello et al., 1976; Ru-fu et al., 1984; Tannenbaum and Correa, 1985). See also Table VI.

Atrophic gastritis is a relevant factor in determining the gastric nitrite level, because nitrate administered to subjects with this type of gastritis results in a ten times higher nitrite concentration than that found in subjects with a normal mucosa (see 1.1.3). A given nitrate dose may be harmless to normal subjects, but noxious to a patient with atrophic gastritis, especially in the presence of precursors of N-nitrosamines or nitrosamides in the diet (see 1.3).

According to Ruddell et al. (1978) iron deficient patients with gastric lesions and patients with pernicious anaemia (PA) are predisposed to stomach cancer and also have a high reduction rate of nitrate to nitrite. The reduction rates in PA patients were nearly fifty-fold higher than of the matched controls, as was the number of bacteria (Ruddell et al., 1978; Reed et al., 1981b).

1.4.3 Summary

The lowest lethal dose of nitrate mentioned in the literature is 80 mg NO_3^-/kg b.w. for adults. Based on more recent data a more realistic lethal dose for adults is around 20 grams or 330 mg NO_3^-/kg b.w. The toxicity of nitrate in man, however, depends on the conversion of nitrate to nitrite. The lowest acute oral lethal dose of nitrite reported for man varied from 33-250 mg NO_2^-/kg b.w., in which the lowest figures applied for children and elderly people. Nitrite is also more toxic to young infants (-3 months) than to adults, giving rise to relatively higher methaemoglobin levels in the blood. The lowest toxic dose reported was 1 mg NO_2^-/kg b.w., whereas in another study 0.5-5 mg NO_2^-/kg b.w. did not cause any toxic effect.

Patients with achlorhydria and/or gastric lesions, and infants up to 3 months of age have to be distinguished as special risk groups for the toxicity of nitrate. Assuming a 80% reduction of nitrate to nitrite, and using the formation of 10% or more methaemoglobin as toxicity criterion, a lowest oral toxic dose of 1.5-2.7 mg NO_3^-/kg can be calculated for this risk group. In reported cases of methaemoglobinemia in infants the intake of nitrate was higher (37.1-108.6 mg NO_3^-/kg b.w.). Of all reported cases of methaemoglobinemia in infants, 97.7% occurred in areas with a nitrate content in drinking water of more than 10-20 mg $\text{NO}_3^- \text{N.l/l}$ (equal to 44.3-88.6 mg $\text{NO}_3^- \text{l/l}$; WHO, 1984; 1985). However, cases of methaemoglobinemia in infants are difficult to evaluate in relation to nitrate intake because of often simultaneously occurring bacterial contamination of drinking water, and bacterial infections in infants, which may influence the reduction of nitrate to nitrite as well as the endogenous synthesis of nitrate. For example, Hegesh and Shiloach (1982) found a significantly increased nitrate blood content, correlated with an increased methaemoglobin content, in infants with acute diarrhea, whereas the intake of nitrate and nitrite in these infants was low (2-7 mg.day⁻¹).

In one experimental study nitrate was given to infants in two different doses. Oral treatment of 11 days to 11 months old healthy infants with 50 or 100 mg NO_3^-/kg b.w. for up to 18 days revealed methaemoglobin concentrations of 5.3-7.5% without cyanosis. Administration of 100 mg NO_3^-/kg b.w. to 6-7 week old babies recovering from previous methaemoglobineamia led to 11% methaemoglobin in combination with cyanosis (Cornbatt and Hartmann, 1948). Control data were not given in this study; normal blood methaemoglobin values are approximately 1-2% (WHO, 1985).

For adults no information on short-term or long-term toxicity is available except for the epidemiological studies on the relation between nitrate or nitrite intake and tumour incidences.

In various countries a relation between high levels of nitrate intake and gastric cancer was reported, and nitrate exposure has been associated with gastric cancer in areas of Chile, Colombia, Denmark, England, Hungary, Italy and Japan (Hartmann, 1983). However, none of the evidence is more than suggestive, and some have even been contradicted by other studies (Davies, 1980; Armijo et al., 1981a and b). Several studies showed a negative association.

In most epidemiological studies, nitrate intake usually was calculated from

questionnaire data or the salivary, gastric or urinary nitrate levels were used as a measure for the nitrate uptake. In several studies, however, a total lack of correlation between gastric cancer risk and salivary nitrate was found, although there was a correlation with the urinary nitrate (Cuello et al., 1976; Eisenbrand et al., 1980).

In the epidemiological and related studies many complicating factors have to be taken into account. Very often the complete information on these factors is not available. In none of the studies summarized in this chapter and in Table V and VI, information about the presence of preformed carcinogenic N-nitroso compounds in the diet or other life style factors was included. Moreover, the relevance of current -exogenous and salivary-nitrate levels to current gastric cancer incidences is disputable, because of the long induction period of tumors.

Possibly other factors than nitrates, responsible for the degenerative changes in the protective gastric mucosa before damage by carcinogens, may be more important. Differences between countries like the consumption of salted or fermented foodstuffs in high gastric cancer mortality countries like Japan and China may play a decisive role in this process and may be an explanation for the inconsistent results in the studies.

In conclusion, no firm relationship was found between the estimated nitrate uptake and gastric cancer in normal healthy individuals, although in several studies an association was indicated. In occupational situations no increased gastric cancer incidences were found after exposure to nitrate or nitrite.

Individuals with stomach lesions or disorders, especially those with atrophic gastritis, patients with pernicious anaemia, and persons on cimetidine and antacida medication present special risk groups in which a correlation between nitrate or nitrite intake and the incidence of gastric cancer cannot be excluded.

Both for healthy individuals and those of the special risk groups, the possible correlation between the nitrate or nitrite intake and gastric cancer is based on the possible endogenous formation of carcinogenic N-nitroso compounds. Combining the facts that for this formation also amine or amide have to be present in relevant concentrations as already discussed in 1.3 and that both nitrate and nitrite were not carcinogenic in animal studies under well-defined conditions, it can be concluded that nitrate and nitrite themselves have no carcinogenic properties.

2. HUMAN TOXICITY - NITROSAMINES

2.1 General introduction

Man may be exposed to preformed nitrosamines in the environment via ingestion (food, snuff dipping and chewing of smokeless tobacco), inhalation (tobacco) and dermal contact (cosmetics). In general nitrosamines occur in the environment in trace amounts. An exception is nitrosodiethanolamine which was found in cosmetics at levels up to 48000 µg/kg and the nitrosamines found in smokeless tobacco used for snuff dipping and chewing (Bartsch and Montesano, 1984)(see Table VII).

In addition nitrosamines may be formed endogenously in the body from various nitrosatable compounds. Nitrosamines formed endogenously from nitrite ingestion via cured meat, provide only a small portion of the total exposure of the general population to nitrosamines from all sources (NAS, 1981; Brunneman et al., 1982-1983).

In this chapter carcinogenic and mutagenic properties of 14 nitrosamines, detected in food, cosmetics and tobacco, will be described. Because of the extensive literature on nitrosamines, especially on dimethyl- and diethylnitrosamine, literature citations are not exhaustive and some reviews are referred to. An on-line literature search was carried out for the more recent data (literature search for mutagenicity and carcinogenicity data for the period Jan 1981-Dec 1985).

- 1) Data concerning carcinogenicity testing have been summarized, using the following criteria:
 - a) an overview of animal species, in which tumours are induced, the target organs and the type of the induced tumours is given.
 - b) (dose-response) studies with experimental animals in which the low(est) doses are used, preferably via the route by which man is exposed, are reported.
 - c) in absence of studies under b) other available studies are mentioned.
- 2) The data concerning mutagenicity testing have been selected using the following criteria:
 - a) The activity of nitrosamines in a variety of test systems, detecting different endpoints, in vitro as well as in vivo, and both in somatic and in germ cells.
 - b) The structure-activity relationship of nitrosamines in the same test

system.

- c) The influence of the type of metabolic activation system on the activity in the in vitro tests and the sensitivity of the target organ(s) in the in vivo tests.
- 3) Nitrosamines are acutely toxic to man at levels that are much higher than those normally encountered in the environment (NAS, 1981)(see 2.4.1). Detailed information on results of acute and/or short-term toxicity experiments in animals will not be given because of the high doses used in these studies and the short treatment times compared to the life-time exposure and the low levels of exposure of man.

2.2 General remarks on carcinogenicity

2.2.1 In general

Nitrosamines were found to be carcinogenic in a large number of animal species (see Table IX). A peculiarity of nitrosamines is the high degree of organ and cell specificity in inducing tumours (Montesano, 1981). The organotropism of the carcinogenic action may be related to a variety of organs and organ systems (see Table X). It does not only depend on the chemical structure of the molecule but also on the daily dose applied and on the strain within the same animal species (Schmahl and Habs, 1980). The high degree of specificity to induce tumours in a given species or organ, appears to be determined by various factors:

- a) the formation of a reactive intermediate
- b) the specific type of DNA damage induced
- c) the level and accuracy of DNA repair processes
- d) the rate of DNA synthesis

In most cases the route of application does not play a decisive role in organotropism of nitrosamines. Nitrosamines have to be regarded as "multipotent" carcinogens. It is highly probable that most nitrosamines also act as a carcinogen to human beings. Predictions as to the organotropism of the carcinogenic action in man cannot be made (Schmahl and Habs, 1980; Montesano and Hall, 1984).

Some N-nitroso compounds induce cancer in the offspring of mother animals treated during pregnancy. The timing of treatment during gestation is a critical factor for the induction of tumours in the offspring although some overlapping periods exist. N-Nitroso compounds induce an embryotoxic effect

in rats when administered between days 1 and 10, a teratogenic effect between days 9 and 16 and a carcinogenic effect from day 10 to delivery. Metabolic competence and the state of differentiation of the various embryonic tissues seem to be the two factors mainly responsible for this different susceptibility during gestation. The failure to induce tumours in offspring with the administration of carcinogens requiring metabolic activation during the first period of pregnancy, may be due to the lack of an adequate metabolic system in the fetus at that time (Bartsch and Montesano, 1976).

2.2.2 Mechanism of activation

Nitrosamines require metabolic activation to reactive intermediates to develop their biologic effects. The liver shows a higher capacity than extrahepatic tissues for metabolizing nitrosamines. Liver of Syrian golden hamsters showed the highest rate of metabolism followed by rat liver, human liver slices have the capacity to activate dimethylnitrosamine that is close to that observed in rat liver. Lower activities were observed in liver of trout and monkeys (Bartsch and Montesano, 1984).

The generally accepted primary reaction, involved in metabolic activation of nitrosamines, is alpha-hydroxylation by P-450 cytochrome requiring enzymes and subsequent dealkylation of these reactive intermediate to an alkyl-diazo-hydroxide and finally the formation of an alkylating agent resulting in alkylation of cellular macromolecules such as proteins, RNA and DNA (see Figure II) (Michejda et al., 1982; Preussman, 1984).

However another pathway, possibly dependent on amine oxidase, may also be involved (NAS, 1981). Pretreatment of animals with compounds known to induce the MFO system, not only failed to increase but actually protected against dimethyl- or diethylnitrosamine induced hepatotoxicity and carcinogenicity. In vitro and in vivo studies revealed that all of the compounds found to inhibit dimethylnitrosamine demethylase activity at concentrations that had little effect on MFO enzymes, were also inhibitors of monoamine oxidase (MAO) activity (Gangolli, 1981).

The important role of alpha-hydroxy-N-nitrosamines as the carcinogenic compounds, has been confirmed by biological investigations of esters of alpha-hydroxynitrosamines which easily decompose by hydrolysis to the free hydroxyderivatives.

Alkylation of DNA leads to the formation of stable adducts such as

alkylated purine and pyrimidine bases. This alkylation of genetic material is probably the initiating event in carcinogenesis (Michejda et al., 1982; Preussman, 1984). Alkylation can occur at at least 12 sites within the DNA molecule (see Figure III). The major alkylated base produced is N7-alkylguanine but it is suggested that alkylation of the oxygen atom may be the critical reaction because a correlation between the production and persistence of O6-alkylguanine and the occurrence of tumours in a variety of organs of rodents exposed to N-nitrosocarcinogens has been observed (NAS, 1981; Pegg, 1980, 1983).

The rate of loss of e.g. O₆-alkylguanine from DNA of various organs of the same animal species or from the same organ of different animal strains or species, differs (Montesano et al., 1980; Setlow et al., 1984). Pegg (1980) stated that the highest activity in removing O6-alkylguanine from DNA is found in the liver. Human liver samples were 10x more active than rat liver samples in catalyzing the removal of O6-methylguanine from DNA (Pegg et al., 1982). Animal studies indicated that there was an enzymatic pathway leading to the loss of O6-alkylguanine from liver DNA and that this pathway was more efficient at lower doses (Pegg, 1977, 1978; Pegg and Hui, 1978).

Recent data provide some evidence of the relative contribution of O2- and O4-alkylthymine to the initiation of the carcinogenic process by nitrosamines (Scherer, et al., 1980; Swenberg et al., 1984)

Determination of alkylated phosphotriesters in rat liver DNA has shown that these phosphotriesters are lost very slowly from DNA, especially after repeated small doses of nitrosamines (Shooter, 1978; O'Connor, 1981). In particular ethylphosphotriesters, produced after treatment with diethylnitrosamine, were lost more slowly from DNA than methylphosphotriesters produced after treatment with dimethylnitrosamine (Den Engelse and Philipppus, 1977; Den Engelse et al., 1983).

The biotransformation and metabolic fate of dialkylnitrosamines higher than diethylnitrosamine is considerably more complex. As secondary reaction beta-hydroxylation of dialkylnitrosamines to methyl-alkyl or dimethylnitrosamines, which then react as the methylating agent, is suggested (Lai and Arcos, 1980; NAS, 1981). Michejda et al. (1979) proposed that conjugates of beta-hydroxydialkylnitrosamines may be directly acting alkylating agents.

The principal metabolic products of dibutylnitrosamine are known to result from beta-, gamma- and omega-oxidation. Metabolites from gamma- and omega-

oxidation have been identified. Products of alpha-hydroxylation of dibutylnitrosamine have not yet been demonstrated in vivo. In contrast alpha-hydroxylation of dibutylnitrosamine was the predominant metabolic pathway in vitro (Okada, 1984). Pathways for the biotransformation of dibutylnitrosamine are given in Figure IV (Olajos, 1977; Schut and Castonguay, 1984).

In general cyclic nitrosamines are metabolised by ring-hydroxylation and also alpha-hydroxylation is the major route of metabolic activation. Beta- and gamma-hydroxylation have been demonstrated, too (see Figure V) (Gilbert et al., 1982, 1984; Hecht et al., 1982).

2.2.3 Detoxication pathways

The existence of detoxication pathways apart from the metabolic activation, was reported (Preussmann and Stewart, 1984; see Figure II). In vitro incubation of dimethylnitrosamine and nitrosomorpholine with rat liver microsomal fractions, demonstrated reduction of the nitrosamine bond to the corresponding hydrazine (Suss, 1965; Grilli and Prodi, 1975; as cited in Preussmann and Stewart, 1984).

Appel et al. (1984) revealed formation of nitrite after incubation of nitrosamines with microsomal systems. Inhibition of microsomal monooxygenase decreased the nitrite generation. It was concluded that denitrosation of N-nitrosamines is a cytochrome P-450 dependent process. Thus cytochrome P-450 dependent monooxygenase catalyses both activation and deactivation of nitrosamines and the balance between activation and deactivation seems to depend on differences between the various forms of P-450 in a given target tissue.

2.3 Animal experiments

2.3.1 Carcinogenicity

Dialkylnitrosamines

The carcinogenic activity of the symmetrical nitrosamines varied considerably. The marked difference in activity between dimethylnitrosamine (DMN) which induces angiosarcoma of the liver in rats and diethylnitrosamine, which at comparable doses, induces both hepatocellular carcinomas and oesophageal tumours, leads to the conclusion that if

alkylation of macromolecules is involved in carcinogenesis by nitrosamines, ethylation is more effective than methylation. As the chain length increases beyond ethyl, the carcinogenic potency of the nitrosamine falls, so that dipropylnitrosamine is less potent than diethylnitrosamine and dibutylnitrosamine is much less potent still, although it gives rise to some bladder tumours as well as tumours of the liver and oesophagus in rats (Lijinsky, 1982).

Dimethylnitrosamine is carcinogenic in all animal species tested (17 species, see Table IX). It induces benign and malignant tumours following administration by various routes including ingestion and inhalation in various organs in various species. DMN produces tumours mainly of the liver, kidneys and respiratory tract. It is carcinogenic in offspring following prenatal exposure (IARC, 1978; Anderson et al., 1982).

In a number of studies dose-response relationships were established. Several tests for low dose effects of DMN have employed the rat, with liver tumours as endpoint.

Terracini et al. (1967) administered 2-50 mg DMN/kg of feed to (Porton) rats for periods of 2 years or longer (0.1-2.5 mg/kg of b.w.). Even at the lowest dose level liver tumours were observed.

In a study reported by Lijinsky and Reuber (1984b) groups of 20 female F344 rats received orally, 5 days/week, for 30 weeks, 5.5 and 13 mg DMN/l of drinking water (about 0.275 and 0.65 mg/kg b.w., respectively). The rats were allowed to die naturally. At 5.5 mg/l mortality occurred after week 70 and at 13 mg/l after week 40. At 13 mg DMN/l liver cell carcinomas (10/20), haemangiosarcomas in the liver (7/20) and neoplastic nodules in the liver (2/20) were observed. 1/20 Rats showed a carcinoma in the oesophagus and 1/20 a carcinoma in the forestomach. In 2/20 rats lung tumours were observed. At 5.5 mg DMN/l, 9/20 rats developed liver cell carcinomas. No haemangiosarcomas in the liver were seen at this dose-level. 5/20 Rats revealed neoplastic liver nodules. In 1/20 rats a lung tumour was observed. No carcinomas in oesophagus or forestomach were seen. In the control group, 2/20 rats showed neoplastic liver nodules.

Arai et al. (1979) observed tumours (hepatocellular carcinoma and haemangioendothelioma) and nodular hyperplasia in the liver of (Wistar) rats fed diets with doses ≥ 1 mg DMN/kg (≥ 0.05 mg/kg b.w.) for a period of 96 weeks. Rats on the lowest dose level in this study (0.1 mg DMN/kg of feed 0.005 mg/kg b.w.) did not show either increased incidences of tumours

in the liver or increased incidences of nodular hyperplasia. No data on mortality and tumour induction in animals that intercurrently died were given.

In a life-time drinking water study with rats performed by BIBRA (Crampton, 1980; Peto et al., 1984) even 0.132 mg DMN/l in the drinking water of males (5.4 µg/kg b.w.) and 0.264 mg DMN/l in the drinking water of females (19 µg/kg b.w.) caused a significantly increased incidence of liver tumours. Even at the lowest levels in this study (0.066 mg DMN/l 2.7 µg/kg b.w. for males and 4.7 µg/kg b.w. for females; 0.033 mg DMN/l 1.4 µg/kg b.w. for males and 2.4 µg/kg b.w. for females) already increased incidences of hyperplastic liver nodules were observed. However no details on tumour incidences were available.

Diethylnitrosamine (DEN) is carcinogenic in all animal species tested (30 species, see Table IX). It induces benign and malignant tumours after its administration by various routes including ingestion, parenteral injection, inhalation and rectal instillation. The major target organs are the liver, respiratory tract, upper digestive tract and kidneys. It is carcinogenic to offspring, following prenatal exposure (IARC, 1978).

In a number of studies dose-response relationships were established. In these studies the rat revealing liver and upper gastro-intestinal tract tumours as endpoints, was used.

Oral doses of 1-10 mg DEN/kg b.w. in life-time studies produced tumour yields in the liver of rats approaching 100% (IARC, 1978).

With lower doses (0.1-0.6 mg DEN/kg b.w. via drinking water) squamous-cell carcinomas of the oesophagus were observed in addition to the liver tumours (Druckrey et al., 1963a; Habs and Schmähl, 1981).

In the study reported by Druckrey et al. (1963a) a dose of 0.3 mg/kg b.w. administered via the drinking water for life-time, caused malignant liver tumours in 62/67 rats. In all rats in this group (67) malignant tumours in liver and/or oesophagus were seen. A dose of 0.15 mg/kg b.w. caused malignant tumours in the liver of 18/30 rats; 27/30 rats showed malignant tumours in liver and/or oesophagus. The mean survival time amounted to 750 days. At the lowest dose-level, i.e. 0.075 mg/kg b.w., 20/60 rats lived longer than 600 days (40/60 died from pneumonia). At 750 days 16 out of these 20 rats have died of which 1/16 showed a benign hepatoma, 3/16 showed multiple papillomas of oesophagus and 3/16 a squamous cell-carcinoma of the nasal cavity. 4 Rats that lived at least upto 850 days showed liver

tumours, 3/4 hepatomas and 1/4 a hepatocellular carcinoma.

In a study reported by Lijinsky et al. (1981a), groups of 20 female F344 rats received dose levels of 0.45-113 mg DEN/l in the drinking water (0.0225-5.7 mg/kg b.w.) for periods up to 104 weeks, 5 days/week. At the lowest dose level (0.45 mg DEN/l) a significantly increased incidence of hepatocellular carcinomas (6/20), hyperplastic liver nodules (5/20), and upper gastro-intestinal tract tumours (6/20 carcinomas + papillomas) was seen after 60 weeks of treatment. After 30 weeks on this dose level already a significantly increased incidence of liver nodules was observed (5/20). In the control group 1/20 hyperplastic liver nodule was seen. Haemangioendothelial sarcomas in the liver were common only among rats receiving the highest concentration. At lower concentrations only hepatocellular carcinomas were seen. Rats with hepatocellular carcinomas also developed hyperplastic nodules and hyperplasia in the liver. At the lowest doses tumours of the upper gastro-intestinal tract were frequent.

In a life-time drinking water study in rats performed by BIBRA (Crampton, 1980; Peto et al., 1984) even 0.132 mg DEN/l in the drinking water of males (5 µg/kg b.w.) and 0.264 mg DEN/l in the drinking water of females (18 µg/kg b.w.) caused a significantly increased incidence of liver tumours. At the lowest levels in this study i.e. 0.066 mg DEN/l (2.5 µg/kg b.w. for males and 4.4 µg/kg b.w. for females) and 0.033 mg DEN/l (1.3 µg/kg b.w. for males and 2.2 µg/kg b.w. for females) no significant induction of liver tumours was seen. However, no details on tumour incidences were available.

Dipropylnitrosamine (DPN) is carcinogenic in rats after oral administration and in rats and hamsters after subcutaneous injection. It produces benign and malignant tumours of the liver, kidneys, oesophagus and respiratory tract (IARC, 1978). It is carcinogenic to offspring, following prenatal exposure (Althoff et al., 1977).

Lijinsky and Reuber (1983) observed increased incidences of carcinomas in liver, oesophagus and nasal cavity of Fischer rats at oral doses of 5.5 and 11 mg DPN/kg b.w. administered by gavage in corn oil, twice a week, for 30 weeks. All rats died within 70 and 40 weeks at 5.5 and 11 mg/kg b.w., respectively.

A dose of 4.5 mg DPN/kg b.w. given to Sprague-Dawley rats via the drinking water for 30 weeks, induced carcinomas in the liver, papillomas and carcinomas in the oesophagus and adenocarcinomas in nasal turbinates. All rats died within 60 weeks (Lijinsky and Taylor, 1979).

Also Druckrey et al. (1967) found in a dose-response study with BD rats induction of liver carcinomas at doses of 4-30 mg DPN/kg b.w. administered via the drinking water. At doses of 4-15 mg DPN/kg b.w. in addition papillomas and carcinomas in oesophagus and tongue were found.

In a study reported by Lijinsky and Reuber (1981a) groups of rats (Fischer strain) received 2.25 mg DPN/kg b.w. via the drinking water, 5 days per week, for 30 weeks. All treated rats (20) died within 40 weeks with oesophagus carcinomas. In addition 12/20 rats showed forestomach tumours, 6/20 tongue tumors and 1/20 revealed a tumour of the oropharynx. No liver tumours were observed in this study.

Dibutyl nitrosamine (DBN) is carcinogenic in all species tested: mice, rats, Syrian golden, Chinese and European hamsters, rabbits and guinea pigs. It produces benign and malignant tumours in the urinary bladder, oesophagus, liver, respiratory tract, stomach and intestine and also leukaemia; it is particularly effective as bladder carcinogen. It is carcinogenic to offspring, following prenatal exposure (IARC, 1978).

Druckrey et al. (1962 as cited in IARC, 1978; , 1967) observed in rats at doses of 10, 20 and 37.5 mg DBN/kg b.w., administered via the diet, increased incidences of liver carcinomas, carcinomas in oesophagus and pharynx and squamous-cell carcinomas in the bladder. At the lower dose levels the incidences of liver carcinomas was lower, but malignant tumours in oesophagus and pharynx were found.

Okajima et al. (1970; as cited in IARC, 1978) administered to rats 0.01 and 0.05% dibutyl nitrosamine in the drinking water (5 and 25 mg/kg b.w.). Bladder tumours were found at both dose levels. At the highest level in addition liver carcinomas were seen. No details on tumour incidences were available.

In a study reported by Lijinsky and Reuber (1983) an oral dose of about 13.5 mg DBN/kg b.w., in corn oil by gavage, was administered, twice a week during 30 weeks, to a group of 20 male F344 rats. The rats were allowed to die naturally. Survival after 100 weeks amounted to 40% compared to 65% in control group. Carcinomas in liver (12/20), forestomach (10/20), lung (9/20), oesophagus (2/20), bladder (7/20) and nasal cavity (2/20) were found. At controls no carcinomas in these tissues were found (only neoplastic liver nodules in 5/20 rats).

Cyclic nitrosamines

Although considerable study of the metabolism of these cyclic compounds has been done, especially in rats, very little indication of the mechanisms by which they induce tumours exists. Notably their binding to DNA in vivo is orders of magnitude smaller than that of the simple aliphatic nitrosamines even in the case of those cyclic nitrosamines that induce liver tumours. They are metabolized at different rates in the rat, but this seems insufficient to explain their markedly different carcinogenic potencies (Lijinsky, 1983). Nitrosopyrrolidine is a much weaker carcinogen than nitrosopiperidine. Nitrosomorpholine is somewhat less potent than nitrosopiperidine (Lijinsky, 1984).

N-Nitrosopiperidine (NPIP) is carcinogenic in mice, rats, Syrian golden, Chinese and European hamsters and in monkeys. It produces benign and malignant tumours of the liver, lung, forestomach and oesophagus in mice, of the liver, oesophagus and respiratory system in rats and of the upper digestive tract, respiratory system, colon and liver in hamsters; it produces hepatocellular carcinomas in monkeys (IARC, 1978; Ketkar et al., 1983a). It is carcinogenic to offspring of hamsters following prenatal exposure (IARC; 1978; Althoff and Grandjean, 1979).

In a study performed by Druckrey et al. (1967), an oral dose of 5 mg NPIP/kg b.w., administered via the drinking water, induced oesophageal tumours in 9/10 BD rats (8 carcinomas, 1 papilloma) and malignant liver tumours in 3/10 rats. The average induction time amounted to 280 days.

A group of 14 male and 15 female Sprague-Dawley rats received, 5 days/week, 100 mg NPIP/l in the drinking water (about 5 mg/kg b.w.). Because of the high mortality, treatment was terminated after 44 weeks. All rats died within 60 weeks. Squamous cell tumours and olfactory carcinomas in nasal turbinates and tumours in the upper gastro-intestinal tract were observed. Hepatocellular carcinomas were found in 1 male and 1 female rat. Haemangioendothelial sarcomas in the liver of 1 male and 3 females were observed. No data on control animals were provided (Lijinsky and Taylor, 1975).

Lijinsky and Reuber (1981b) observed at the same dose level in drinking water, given for 5 days/week during 28 weeks to female F344 rats, induction of benign and malignant tumours in the oesophagus. In addition one rat showed a forestomach papilloma, one a forestomach carcinoma and one a larynx carcinoma. No liver tumours were seen in this study. All rats died

within 36 weeks.

In a study reported by Eisenbrand et al. (1980) male and female Sprague-Dawley rats received 5 days/week for life-time 0, 0.024, 0.12, 0.6 and 3.0 mg NPIP/kg b.w. via the drinking water. Median survival time amounted to 620, 812, 746, 795 and 392 days, respectively. At doses ≥ 0.12 mg/kg b.w. a dose-related induction of benign and malignant tumours in liver (6/75, 16/34 and 11/34 at 0.12, 0.6, and 3.0 mg/kg b.w., respectively) and oesophagus and/or forestomach (2/75, 8/34 and 24/34, respectively) was observed. At the lowest dose level, 0.024 mg/kg b.w., 3/78 animals revealed benign and malignant tumours in the liver, but no tumours in oesophagus and/or forestomach were seen. At the control group (39 males and 39 females) no tumours in liver and oesophagus and/or forestomach were observed.

The lowest dose level inducing tumours was found in a life-time drinking water study in rats performed by BIBRA (Crampton, 1980; Peto et al., 1984). Groups of 6 male and 6 female Colworth rats were given doses of 0.18-92.93 mg NPIP/l in the drinking water. At doses of 0.36 mg/l (15.7 μ g/kg b.w. for males and 27.6 μ g/kg b.w. for females) and higher a definite carcinogenic response was found. No details on tumour incidences were available.

N-Nitrosomorpholine (NMOR) is carcinogenic in mice, rats, Syrian golden, Chinese and European hamsters and in two fish species. Following oral administration it produces benign and malignant tumours of the liver and lung in mice, of the liver, kidneys and blood vessels in rats and of the liver and upper respiratory tract in hamsters. After subcutaneous injection it produces tumours of the upper digestive and respiratory tract in hamsters. It produces liver tumours in fish (IARC, 1978; Ketkar et al., 1983b; Lijinsky et al., 1984).

In a study reported by Lijinsky and Reuber (1982) a dose of 16 mg NMOR/l of drinking water (0.8 mg/kg b.w.), administered, 5 days/week, during 50 weeks, induced hepatocellular carcinomas in 18/20 male (F344) rats, haemangioendothelial sarcomas in the liver of 4/20 rats, papillomas in oesophagus of 2/20 rats, papillomas in the forestomach of 1/20 rats and a papilloma of the tongue in 1/20 rats. All rats died within 110 weeks (Lijinsky and Reuber, 1982).

In a long-term oral study with rats, given 8 mg NMOR/l in drinking water (0.4 mg/kg b.w.) for 30 weeks (Lijinsky et al., 1976), 11/30 (male

Sprague-Dawley) rats developed hepatocellular carcinomas and 1/30 a haemangioendothelioma. The rats were allowed to die naturally. No data on tumours in other organs than the liver were provided.

The lowest tested oral dose in a long-term study with Sprague Dawley rats, amounted to 5 mg/kg of feed (0.25 mg/kg b.w.). The rats were derived from a F1 and F2 generation exposed in utero and the experiment was terminated when surviving rats of F2 generation were 125 weeks of age. Hepatocellular carcinomas (incidence: 58%) and haemangiosarcomas in the liver (incidence: 15%) were observed. In addition 9% of the rats revealed lung angiosarcomas and 22% of the rats revealed metastases from liver to lung. In 1% of the rats angiosarcomas in tissues other than liver of lung were observed. Median age at death with a liver carcinoma amounted to 106 weeks. At the higher dietary level (50 mg/kg of feed) an almost 100% incidence of liver carcinomas was seen (Shank and Newberne, 1976).

N-Nitrosopyrrolidine (NPYR) is carcinogenic in rats and hamsters after oral administration. It produces hepatocellular carcinomas in rats and hepatocellular carcinomas and respiratory tract neoplasms in hamsters. It also increases the incidence of hepatocellular carcinomas and lung adenocarcinomas in mice following oral administration (IARC, 1978; Ketkar et al., 1982; Pearson et al., 1982).

In a number of oral studies with rats (Preussmann et al., 1977; Habs et al., 1980; Lijinsky and Reuber, 1981b; Hoos et al., 1985) induction of hepatocellular carcinomas at doses ≥ 1 mg NPYR/kg b.w. was observed.

In a dose-response study reported by Preussmann et al. (1977), Sprague-Dawley rats received doses of 0, 0.3, 1, 3 or 10 mg NPYR/kg b.w. via the drinking water, for lifetime. At doses ≥ 1 mg NPYR/kg b.w., hepatocellular carcinomas were induced at significantly increased incidences (9/24, 30/38, 13/62, 0/60 and 0/61 in 10, 3, 1, 0.3 and 0 mg group, respectively). Hepatocellular adenomas were seen at 5/24, 1/38, 4/62, 3/60 and 0/61, respectively. At all treatment groups a slight increase in the overall incidence of malignant tumours and leukaemias was found. Median time of death of tumour bearing animals amounted to 444, 533, 685, 664 and 587 days; respectively at 10, 3.0, 1.0, 0.3 and 0 mg NPYR/kg b.w.

A dose-response study in small groups (6 males, 6 females) of Colworth rats was performed by BIBRA (Crampton, 1980; Peto et al., 1984). The dose levels varied from 0.50-287.33 mg NPYR/l of drinking water and were administered for a period of 2.5 years. At doses of 4.49 mg NPYR/l (200 μ g/kg b.w. for

males and 350 µg/kg b.w. for females) and higher a definite carcinogenic response was found. However no data on tumour incidences were given.

Nitrosothiazolidine

No long term experiments for carcinogenic activity of nitrosothiazolidine in experimental animals were available.

N-Nitrosodiethanolamine (NDELA)

Human exposure occurs mainly through cosmetics and tobacco and in certain industrial settings.

Skin penetration of NDELA was demonstrated in animals in vivo by the detection of unchanged NDELA in urine after dermal application (Lijinsky et al., 1981; Marzulli et al., 1981; Preussmann et al., 1981; Lethco et al., 1982; Airoidi et al., 1984).

Edwards et al. (1979a) detected 20% of an applied dose as unchanged NDELA in urine of man after topical application and Bronaugh et al. (1981) reported penetration of the human skin by NDELA with an in vitro technique. Despite the high percentage of excretion of unchanged NDELA in urine of rats after various routes of administration (70-84%) (Preussmann et al., 1978, 1981) a relatively strong carcinogenicity in rats was found in several dose-response studies (IARC, 1978; Lijinsky et al., 1980; Preussmann et al., 1982; Lijinsky and Reuber, 1984a).

NDELA is carcinogenic in rats after oral administration and in hamsters after subcutaneous and dermal application. It produces hepatocellular carcinomas, renal adenomas, squamous-cell carcinomas and neuroepitheliomas in nasal cavity in rats. Adenocarcinomas of nasal cavity, papillary tumours of the trachea, hepatocellular adenomas and local fibrosarcomas in hamsters were observed (IARC, 1978; Preussmann et al., 1982; Hoffmann et al., 1983). On the contrary in mice, given daily 3900-62500 mg NDELA/l of drinking water no tumours were observed. Cholangiofibrosis and cirrhosis, as well as some hyperplastic nodules were found in the livers of many mice receiving the higher doses (as cited in Lijinsky et al., 1980).

Swabbing of the oral cavity of hamsters with 20 mg diethanolnitrosamine in 0.4 ml of a 0.9% NaCl solution, 3 x weekly, for 45 weeks, induced tumours of the nasal cavity and papillomas in the upper respiratory tract. No tumours in oral cavity were seen (Hoffmann et al., 1983).

Topical applications of 25 mg diethanolnitrosamine in 0.5 ml acetone, to

the shaven skin of hamsters, 3 x weekly, for 36 weeks, induced nasal cavity tumours and papillomas in the upper respiratory tract. No skin tumours were observed. Doses of 8 and 2.5 mg, applied topically, did not induce tumours (Hoffmann et al., 1983).

In an oral dose-response study in male Sprague-Dawley rats, with dose levels of 1.5-400 mg NDELA/kg b.w. given in the drinking water, animals were allowed to die naturally or were killed intercurrently, when moribund (Preussmann et al., 1982). Treatment-related benign and malignant tumours in liver (incidence: 31/36, 32/36, 33/36, 43/72, 7/72 and 0/88 at 400, 100, 25, 6, 1.5 and 0 mg/kg b.w., respectively) and nasal cavity (incidence: 1/36, 6/36, 6/36, 0/72, 2/72 and 0/88, respectively) were seen. Induction of hepatocellular carcinomas was clearly dose-related. Low doses also induced benign neoplasms in the liver. Other liver tumours were of mesenchymal and ductal origin and nasal cavity neoplasms were diagnosed as squamous-cell carcinomas and neuroepithelioma of the olfactory epithelium. Even the lowest dose i.e. 1.5 mg NDELA/kg b.w. induced benign and malignant hepatocellular tumours and squamous-cell carcinomas and neuroepitheliomas in the nasal cavity. No liver tumours or tumours in nasal cavity in the control group were seen (Preussmann et al., 1982). Long-term oral studies with doses lower than 1.5 mg NDELA/kg b.w. were not available.

Tobacco specific nitrosamines (TSNA)

Of the tobacco specific nitrosamines (TSNA), 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK) appears to be the most important because of its strong carcinogenicity.

In a subcutaneous dose-response study in F344 rats, NNK and N-nitrosonornicotine (NNN) were about equipotent in inducing nasal cavity tumours: however with the lowest dose studied (1 mmol/kg total) NNK also caused a high incidence of lung tumours and some liver tumours (Hoffmann et al., 1984).

On basis of literature it can be estimated that carcinogenicity of NNK in Syrian golden hamsters is more pronounced than that of N-nitrosomorpholine and could be equivalent to that of N-nitrosodiethylamine (Hecht et al., 1984).

At oral doses equivalent to those at which NNN induced tumours of the oesophagus or nasal cavity, N-nitrosoanabasine (NAB) did not show a significant tumorigenic activity in F344 rats (Hoffmann et al., 1975),

although higher doses of NAB did induce oesophageal tumours (Boyland et al., 1964). At equimolar subcutaneous doses, NNN induced tumours in nasal cavity, lung and liver of F344 rats while NAT did not show a tumorigenic activity (Hoffmann et al., 1984).

The route of administration has some effect on target tissue specificity of TSNA.

NNK induced carcinomas of nasal cavity, lung and liver in rats after subcutaneous injection with a clear dose-response relationship. In Syrian golden hamsters it induced benign and malignant tumours of the nasal cavity, trachea and lung after subcutaneous injection, even after a single treatment. Intraperitoneal injection in mice induced benign and malignant tumours of the lung.

Long-term oral or inhalation studies were not available (IARC, 1985).

Oral swabbing in Syrian golden hamsters caused induction of tumours in nasal cavity and lung (Rivenson et al., 1983, as cited in Hecht et al., 1984).

N-Nitrosornicotine (NNN) produced carcinomas of the upper digestive tract, mainly the oesophagus, and carcinomas of the nasal cavity in rats after oral administration. In hamsters after oral administration carcinomas of nasal cavity and papillomas of the trachea were observed. Following subcutaneous administration N-nitrosornicotine primarily produced tumours of the nasal cavity in rats and tumours of the trachea in hamsters. Intraperitoneal injection produced lung tumours in mice and nasal cavity and tracheal tumours in hamsters (IARC, 1985).

Oral swabbing in Syrian golden hamsters caused induction of tumours in trachea and nasal cavity (Rivenson et al., 1983, as cited in Hecht et al., 1984).

At a dose of 120 mg NNN/l in drinking water (6 mg/kg b.w.), given for 36 weeks to a group of 12 male and 12 female F344 rats, all rats died within 12 months. Benign and malignant tumours in oesophagus and nasal cavity were induced (Hecht et al., 1983b).

In a study reported by Castonguay et al. (1984) a group of 30 male F344 rats (13 weeks old) received daily, 7 days/week, for 27 weeks, 17.5 mg NNN/l of liquid diet (about 2.5 mg/kg b.w./day). The rats were killed when moribund or at 98 weeks of age (mean survival time 83 weeks). 18/30 Rats revealed nasal cavity tumours (11 benign tumours, 2 esthesioneuroepitheliomas, 5 squamous cell carcinomas), 25/30 rats

oesophageal tumours (16 benign tumours, 9 squamous cell carcinomas), 2/30 rats benign tumours of the tongue and 1/30 rats a squamous cell carcinoma in the lungs. In a control group of 20 rats, receiving liquid diet and additionally 3x weekly a s.c. injection with 0.9% NaCl, no such tumours were found.

N-Nitrosoanabasine (NAB) induced oesophageal carcinomas and/or papillomas in rats after oral administration (IARC, 1985).

Oral administration of NAB via the drinking water (0.02%) for 30 weeks, 5 days/week, to male Fischer rats caused induction of an oesophageal papilloma in 1/17 rats and a pharyngeal papilloma in 1/17 rats. Rats were killed when moribund or at 11 months (Hoffmann et al., 1975). (note the short treatment and observation time and the low dose used. As cited in IARC, 1985).

Subcutaneous treatment of Syrian golden hamsters did not reveal increased tumour incidences (Hilfrich et al., 1977). (note the short treatment time and small number of animals)(IARC, 1985).

In a study, reported by Boyland et al. (1964), administration of 0.2% NAB in the drinking water of rats (Chester Beatty)(5 mg/rat) induced oesophageal carcinomas in 4/16 males and 1/14 females, and oesophageal papillomas in 9/16 males and 11/14 females after 347-521 days. In the control group (16 males and 16 females) only 1 malignant lymphoma was seen.

N-Nitrosoanabatine (NAT) given subcutaneously to F344 rats (total dose 1.0, 3.0, and 9.0 mmol/kg b.w.) did not cause an induction of tumours (Hoffmann et al., 1984). The study was considered to be inadequate for evaluation of the carcinogenicity because of the short treatment time and the small number of animals used (IARC, 1985).

4-(N-nitrosomethylamino)-4-(3-pyridyl)butanal (NNA) was administered intraperitoneally 3 x weekly for 7 weeks to female A/J mice for a total of 22 injections. The dose used was 1 mg/mouse given as an 0.5% solution in saline (0.2 ml/injection). The mice were observed for an additional 30 weeks. 9/25 Mice showed lung adenomas compared to 3/25 in vehicle controls and 1/25 in untreated controls (Hecht et al., 1978). The study was considered to be inadequate for evaluation of the carcinogenicity (IARC, 1985).

2.3.2 Mutagenicity

A general overview on the activity of the nitrosamines in the tests is given in Table VIIIa and VIIIb.

Dialkylnitrosamines

In vitro studies

In bacterial assays with *Salmonella typhimurium* and *Escherichia coli* using the standard plate-test with a metabolic activation system (rat liver S-9), only a weak response or no response at all with dialkylnitrosamines was observed in strains detecting base-pair substitutions (McCann et al., 1975; Rao et al., 1979a, 1981b; Araki et al., 1984; Phillipson and Ioannides, 1985); liquid preincubation strongly enhanced the mutagenic activity (Yahagi et al., 1977; Prival et al., 1979; Rao et al., 1979a; Andrews and Lijinsky, 1980; Araki et al., 1984). At preincubation of *Salmonella typhimurium* with dialkylnitrosamines and liver S-9 in a weakly acidic medium, a higher mutagenic activity was found than at preincubation in a neutral medium (Negishi and Hayatsu, 1980; Guttenplan, 1984). Marked species differences in bioactivation were seen, the Syrian hamster in contrast to the rat being a very effective activator (Prival and Mitchell, 1981; Lijinsky and Andrews, 1983; Phillipson and Ioannides, 1985). Hepatocyte-mediated metabolic activation appeared to be more effective than activation by liver homogenate fractions (Raineri et al., 1981; Rumruen and Pool, 1984). Czygan et al. (1973) reported activation of DMN by a microsomal fraction of human liver, but Bartsch et al. (1976) and Phillipson and Ioannides (1985) detected no activation of DMN, DPN, and DBN by human liver S-9 fractions. Yahagi et al. (1977) found a direct relationship between mutagenic activity and toxicity of dialkylnitrosamines in bacterial assays and the length of the alkyl chain. Andrews and Lijinsky (1980) and Rao et al. (1979a) reported an inverse relationship between the mutagenic potency of dialkylnitrosamines in the *Salmonella* assay and the carcinogenic potency in animal studies.

In fungi DMN, DEN and DPN induced mutations in the presence of an exogenous metabolic activation system (Montesano and Bartsch, 1976; Sankaranarayanan, 1981; Frezza et al., 1983). In *Saccharomyces cerevisiae* D7 cells in the logarithmic growth phase, containing a high level of cytochrome P-450, DMN revealed mutagenic activity without exogenous metabolic activation (Carratore, del et al., 1983). In growth-mediated assays with *Aspergillus*

nidulans and Neurospora crassa DMN and DEN revealed also positive results without exogenous metabolic activation (Whong and Ong, 1979; Bignami et al., 1981). DBN showed a weak induction of mitotic crossing-overs in S. cerevisiae D3 in the presence of Udenfreund's hydroxylation system (Montesano and Bartsch, 1976).

In the plant species Arabidopsis thaliana, Tradescantia and Glycine max but not in Vicia faba or barley, DMN revealed mutagenic activity (Sankaranarayanan, 1981; Gichner et al., 1982; Gichner and Veleminsky, 1984; Tano and Yamaguchi, 1985). DEN showed mutagenic activity in Tradescantia (Tano and Yamaguchi, 1985) but not in V. faba or A. thaliana (Sankaranarayanan, 1981). DPN was not tested in plant systems. DBN did not reveal any mutagenic activity in A. thaliana (Veleminsky and Gichner, 1968).

In mammalian cells in vitro, DMN, DEN, DPN and DBN induced gene-mutations in the presence of various metabolic activation systems (subcellular liver fractions, liver cells or liver perfusion systems of mice and rats) (O'Neill et al., 1977; Jenssen et al., 1979; Bartsch et al., 1980; Jones and Huberman, 1980; Sankaranarayanan, 1981; Amacher and Paillet, 1983). Hamster hepatocytes appeared to be very effective metabolic activators (Amacher and Paillet, 1982; Katoh et al., 1982). The activity of dialkyl nitrosamines to induce gene-mutations in mammalian cells in vitro decreased with increasing length of the alkyl chain (Jones and Huberman, 1980). DMN and DEN induced also gene-mutations in human fibroblasts in vitro in the presence of rat liver S-9, primary rat or human hepatocytes for metabolic activation (Michalopoulos et al., 1981; Chen et al., 1982; Strom et al., 1983).

In mammalian cells in vitro, including human cells, DMN induced chromosomal aberrations in the presence of mouse, rat or human liver microsomal fractions (Matsuoka et al., 1979; Ishidate et al., 1981; Sankaranarayanan, 1981; Thust, 1982). Chromosomal aberrations were induced too by DEN, DPN and DBN in Chinese hamster cells in vitro in the presence of rat liver microsomes (Matsuoka, et al., 1979; Ishidate et al., 1981; Sankaranarayanan, 1981). Aberration types induced by both DMN and DEN were mainly chromatid exchanges, whereas those induced by DPN and DBN were predominantly breaks (Matsuoka et al., 1979).

In mammalian cells in vitro, including human cells, DMN and DEN induced sister chromatid exchanges (SCE's) in the presence of rat liver microsomal

fractions (Okinaka et al., 1981; Sankaranarayanan, 1981; Tomkins et al., 1982; Inoue et al., 1983). Also human liver microsomes were able to convert DMN to a compound inducing SCE's in Chinese hamster V79 cells (Thust, 1982). Manoharan and Banerjee (1985) observed SCE induction in an organ culture of mammary glands of mice, in vitro exposed to DEN without an exogenous metabolic activation system.

In cell transformation assays, DMN showed positive effects (IARC, 1978; Amlacher, 1982). DEN did not induce transformation of Syrian hamster embryo cells, but treatment of the embryo by transplacental administration of DEN led to the appearance of transformed cells in the primary culture (IARC, 1978).

Studies in Drosophila

In Drosophila melanogaster, DMN and DEN induced sex-linked recessive lethals (Nix et al., 1981; Vogel et al., 1981; Woodruff et al., 1984). DMN induced also translocations and loss of chromosomes in Drosophila (Vogel et al., 1981; Woodruff and Brodberg, 1983) while DEN did not induce dominant lethals or chromosome loss and appeared to be a weak inducer of translocations at toxic concentrations only (LD60-LD90) (Vogel et al., 1981). However DEN induced chromosome loss in repair deficient strains of Drosophila (Zimmering and Kammermeyer, 1983). In tests with Drosophila detecting somatic mutations and recombinations DMN, DEN, and DPN showed positive effects (Würgler and Vogel, 1985).

In vivo mammalian studies

DMN induced gene-mutations in somatic cells of mice in a spot test at intraperitoneal doses ≥ 5 mg/kg b.w., albeit at a low incidence (Fahrig et al., 1981).

DMN showed a weak positive effect in a dominant lethal study with mice after s.c. treatment, but no effect was seen after i.p. treatment (Sankaranarayan, 1981).

In a heritable translocation assay in male mice, given orally 0.05 or 0.1 mg DMN/kg b.w./day for 8 weeks, no chromosomal translocations in primary spermatocytes of parents or offspring were induced (Savkovic et al., 1978).

DMN induced chromosomal aberrations in rat peripheral lymphocytes and Chinese hamster liver cells at i.p. doses ≥ 30 mg/kg b.w. (once) and

5000 mg/kg b.w.(once weekly for 6 weeks), respectively (Natarajan et al., 1978; Sankaranarayanan, 1981).

For the induction of micronuclei in bone-marrow cells in vivo by DMN, four studies in mice showed positive results, one study in mice negative results, one study in rats equivocal results and one study in Syrian hamsters positive results after one or two high (compared to LD50) i.p. doses (Jenssen and Ramel, 1978; Trzos et al., 1978; Matter and Wild, 1981; Watanabe et al., 1982). After repeated, i.p. administration of relatively low doses of DMN to mice and hamsters (5 x 6 mg/kg b.w. to mice and 3 x weekly for 1-8 weeks 4.2 mg per hamster) no induction of micronuclei in bone-marrow cells was found (Matter and Wild, 1981). When adult rats before or after i.p. treatment with DMN, underwent partial hepatectomy, to provide an adequate population of dividing cells, a significant induction of micronuclei in hepatocytes was seen (Tates et al., 1983). In transplacental assays with pregnant rats, i.p treatment of the dams with DMN resulted in induction of micronuclei in fetal polychromatic erythrocytes. No induction of micronuclei in maternal bone-marrow cells was seen (Fumero et al., 1981; Suter and Kubli, 1982). Intra-amniotical treatment of the dams with DMN did not reveal any effect in the fetuses (Suter and Kubli, 1982).

Induction of SCE's in vivo was caused by DMN in bone-marrow cells of mice (Sankaranarayanan, 1981; Parodi et al., 1983) and Chinese hamsters (Neal and Probst, 1983) and in peripheral blood lymphocytes of rats (Sankaranarayanan, 1981; Tates et al., 1983). In transplacental assays with pregnant mice, DMN caused a dose-related induction of SCE's in maternal bone-marrow cells and fetal liver cells at i.p. doses of 10-100 mg/kg b.w. (Sharma et al., 1983).

DMN did not induce sperm head abnormalities in mice after 5 i.p. injections (dose unknown) (Bruce and Heddle, 1979).

DEN induced gene-mutations in somatic cells of mice in a spot-test at i.p. doses ≥ 5 mg/kg b.w., albeit at a low incidence (Fahrig et al., 1981).

No gene-mutations in germ cells of mice were detected in a specific locus assay after i.p. treatment with 100 or 150 mg DEN/kg b.w. (Russell and Kelly, 1979).

DEN did not reveal a mutagenic effect in a dominant lethal study in mice after i.p. treatment with 13.5 mg DEN/kg b.w. (Sankaranarayanan, 1981).

No chromosomal aberrations in peripheral blood lymphocytes of rats were

observed after i.p. treatment with 300 mg DEN/kg b.w. (Natarajan et al., 1978). Chromosomal aberrations in hepatocytes were detected by Grover and Fischer (1971) after s.c. treatment of newborn rats with 10 mg DEN/kg b.w. for 3 days, by Hitachi et al. (1974) after oral treatment of rats for 3-5 weeks with 50 mg DEN/l of drinking water and by Horiuchi et al. (1984) at oral treatment of rats before or after partial hepatectomy. When 50-200 mg DEN/kg b.w. was injected s.c. to pregnant hamsters, chromosomal aberrations in fetal tracheal cells, cultured 2 h after treatment, were observed (Tsuda et al., 1983).

No induction of micronuclei in bone-marrow cells in vivo by DEN was observed in two studies in mice and one study in rats after one or two high (compared to LD50) i.p. doses (Trzos et al., 1978; Matter and Wild, 1981). When rats before or after i.p. treatment with DEN underwent partial hepatectomy, a significant induction of micronuclei in hepatocytes was seen (Tates et al., 1983). In transplacental assays with mice and rats, i.p. treatment of the pregnant animals with DEN resulted in induction of micronuclei in fetal liver erythroblasts and polychromatic erythrocytes, respectively. No induction of micronuclei in maternal bone-marrow cells was seen (Suter and Kubli, 1982; Cole et al., 1983). Intra-amniotical treatment of pregnant rats with DEN did not induce this effect in fetuses (Suter and Kubli, 1982).

DEN induced SCE's in bone-marrow cells of mice after an i.p. dose of 216 mg/kg b.w. (Parodi et al., 1983), but in two other studies with mice and Chinese hamsters, respectively (Sankaranarayanan, 1981) no effect was observed. In peripheral lymphocytes of rats, treated i.p. with DEN, Natarajan et al. (1978) did not find an induction of SCE's at 300 mg/kg b.w. while Tates et al. (1983) observed a positive response at 50 mg/kg b.w.

In conclusion, when DMN and DEN were tested in the same system, DEN showed less activity than DMN or no activity at all (Sankaranarayanan, 1981).

DPN was tested for the induction of SCE's in bone-marrow cells of mice. A positive effect was seen at i.p. doses $\geq$ 172 mg/kg b.w. (Parodi et al., 1983).

DBN did not induce chromosomal aberrations in bone-marrow cells of rats (dose and route unknown) (Kawachi et al., 1980) or micronuclei in bone-

marrow cells of mice after i.p. treatment (Bruce and Heddle, 1979). A positive effect was found for the induction of SCE's in bone-marrow cells of mice at i.p. doses ≥ 300 mg/kg b.w. (Parodi et al., 1983).

DBN did not induce sperm head abnormalities in mice after 5 i.p. injections (dose unknown) (Bruce and Heddle, 1979).

Host-mediated assays

In host-mediated assays in mice and rats with bacteria and fungi as indicator organism, DMN showed a mutagenic effect. DEN showed positive and negative results in this test system and when mutagenic activity was observed, it was always lower than the activity induced by DMN, while in vitro assays with the same organisms revealed a higher activity with DEN. In the majority of these host-mediated assays the doses used were relatively high (= toxic) (Sankaranarayanan, 1981; Fahrig and Remmer, 1983).

Intrasanguineous host-mediated assays showed a higher sensitivity compared to intraperitoneal assays. This may be due to a short half-life of the reactive mutagen, which cannot reach the indicator organism in the peritoneum in sufficient amounts from the site of its formation, mainly the liver (Pueyo et al., 1979; Bakshi and Brusick, 1980; Neale and Solt, 1980; Barale et al., 1981; Kerklaan et al., 1981, 1983; Frezza et al., 1983).

In an intrasanguineous host-mediated assay with DMN performed in mice and rats with Neurospora crassa as indicator organism, Whong and Ong (1979) found the highest activity in liver followed by kidney and lung. However in vitro homogenate-mediated assays with N. crassa showed a reversed order; the highest activity was found in the presence of a lung homogenate, followed by the kidney and liver homogenate.

DBN showed a mutagenic response in an intrasanguineous host-mediated assay in rats with Saccharomyces cerevisiae D4 as indicator organism (Fahrig and Remmer, 1983).

In intratesticular host-mediated assays in rats with S. cerevisiae D4 as indicator organism, DMN showed mutagenic activity at a high subcutaneous dose (740 mg/kg b.w.) (Sankaranarayanan, 1981) but DEN and DBN did not show any mutagenic activity indicating that the compounds injected s.c. and i.p., respectively or their metabolites did not reach the testes (Fahrig and Remmer, 1983).

In intraperitoneal host-mediated assays in mice with mammalian cells as

indicator organisms, DMN induced gene-mutations and SCE's. DEN induced SCE's in this system only at repeated i.p. doses of 100 mg/kg b.w. and after pretreatment of mice with PCB (Sirianni and Huang, 1973; O'Neill et al., 1977; Sankaranarayanan, 1981).

Assays for DNA damage

In vitro studies

In tests for indirect DNA damage in bacteria and yeast, measuring differential survival between DNA-repair proficient and DNA-repair deficient strains, DMN and/or DEN showed positive effects in Escherichia coli, Bacillus subtilis and Saccharomyces cerevisiae under conditions of metabolic activation (Kada, 1981; McCarroll et al., 1981a, 1981b; Rosenkranz, 1981; Nestmann et al., 1982; Mamber et al., 1983; Mohn et al., 1983). DEN showed a negative effect in this system with growing cells of S. pombe (Nestmann et al., 1982).

DBN showed a DNA damaging effect in a differential killing assay with E. coli (Pool et al., 1982).

In a prophage induction assay with E. coli in the presence of a hamster liver microsomal fraction, DMN, DEN, DPN and DBN revealed a DNA damaging effect. DEN showed the greatest activity followed by DPN and DMN. DBN showed borderline activity in this assay (Elespuru, 1984).

DMN inhibited DNA synthesis in rat oesophageal epithelium in vitro at high concentrations (10-40 mg/l) without metabolic activation (Mirvish et al., 1978). Inhibition of DNA synthesis in human (HeLa and EUE) cells was caused by DMN in the presence of a metabolic activation system (Bianchi et al., 1982; Painter and Howard, 1982).

Single strand breaks in DNA of Chinese hamster ovary cells were induced by DMN in the presence of a metabolic activation system (Douglas and Grant, 1980). In primary cultures of rat hepatocytes, single strand breaks, but no double strand breaks, were induced by DMN, DEN, DPN and DBN (Schwarz et al., 1980; Bradley and Dysart, 1981; Mendoza-Figueroa et al., 1983; Sina et al., 1983).

In human skin fibroblasts in culture, DMN induced single strand breaks in DNA in the presence of a metabolic activation system (Laishes and Stich, 1973). In primary cultures of human hepatocytes DMN induced single strand breaks, but a large interindividual variation was observed (Martelli et al., 1985).

Induction of DNA repair, as measured by unscheduled DNA synthesis (UDS), was revealed by DMN and DEN in Chinese hamster ovary cells, human fibroblasts and HeLa cells in the presence of a metabolic activation system (Martin et al., 1978; Douglas and Grant, 1980; Sega and Mitchell, 1981; Strom et al., 1981). DMN and DEN induced UDS in primary cultures of hepatocytes of mice, rats, hamsters or humans (IARC, 1978; Williams and Laspia, 1979; Probst et al., 1981; Strom et al., 1981, 1983; McQueen et al., 1983) and in primary cultures of rat tracheal epithelium cells (Ide et al., 1981; Doolittle and Butterworth, 1984). Ide et al. (1982) detected induction of UDS by DMN and DEN in cultures of human oral mucosa indicating metabolic activity of this tissue.

DPN and DBN induced UDS in human HeLa cells in the presence of a metabolic activation system (Martin et al., 1978). DPN showed a positive result for inducing UDS in primary cultures of rat hepatocytes (Probst et al., 1981; Yamazaki et al., 1985). Neither DPN nor DBN induced UDS in a primary culture of rat tracheal epithelium (at the same concentrations a positive effect for DMN and DEN) (Ide et al., 1981).

In vivo mammalian studies

Nuclear DNA synthesis in liver epithelium and/or renal tubular epithelium of mice, treated i.p. with DMN, DEN, DPN and DBN, was inhibited (Amlacher and Rudolph, 1978; Amlacher, 1982).

A 50% inhibition of DNA synthesis was found in oesophageal epithelium of rats, after an i.p. dose of 22 mg DMN/kg b.w. Recovery occurred within 1 day (Mirvish et al., 1978). Oral treatment of rats with 20 mg DMN/l of drinking water, caused inhibition of DNA synthesis in oesophageal epithelium after 2 weeks of treatment (Mirvish et al., 1978).

DNA synthesis in testes of mice treated orally with DMN was inhibited (30% inhibition at 50 mg/kg b.w.) (Friedman and Staub, 1976; Seiler, 1981). DEN and DBN inhibited thymidine incorporation (89 and 56% inhibition, respectively) into testicular DNA of mice treated intraperitoneally with 100 and 500 mg/kg b.w., respectively (Friedman and Staub, 1976).

Single strand breaks (SSB's) in rat liver DNA were induced by DMN, DEN, DPN and DBN at oral doses amounting to 1/5 LD50 and higher (Schwarz et al., 1979; Brambilla et al., 1981; Bermudez et al., 1982). On molar base the DNA damaging potency decreased with increasing length of the alkyl chain and varied over a 28-fold range as measured by an alkaline elution technique

(Brambilla et al., 1981). SSB's in DNA of liver, lung, kidneys and thymus, but not in brain or duodenum were induced after i.p. treatment of mice with doses of 2.3-150 mg DMN/kg b.w. (Parodi et al., 1978) and after i.p. treatment of rats with doses of 10-40 mg DMN/kg b.w. (Petzold and Swenberg, 1978). At low concentrations the DNA damage was far higher in liver than in lung and kidney of mice (Parodi et al., 1978). Induction of SSB's in DNA by DEN was observed in liver, duodenum and kidney of rats, but not in lung, brain or thymus, after i.p. doses of 40-80 mg/kg b.w. (Petzold and Swenberg, 1978). Stewart (1981) found that after i.p. administration of DMN or DEN to rats, SSB's in liver persisted 4 and > 6 days, respectively (as measured by benzoylated DEAE-cellulose chromatography). Also Den Engelse and Philippus (1977) and Floot et al. (1979) found a faster repair of SSB's in rat liver DNA after treatment with DMN than after treatment with DEN at equimolar i.p. doses. Highest amount of DNA damage (SSB's) in rats was found in organs most susceptible to tumour induction (Brambilla et al., 1983; Carlo et al., 1983).

Unscheduled DNA synthesis (UDS) was induced by DMN in various somatic cell cultures (epithelial and endothelial lung cells, liver- and kidney cells, epithelial cells of nasal turbinates and trachea) of mice and rats after oral, s.c or i.p. treatment or after inhalation (Brambilla et al., 1978; Mirsalis and Butterworth, 1980; Sega and Mitchell, 1981; Mirsalis et al., 1982; Tyson and Mirsalis, 1983; Doolittle et al., 1984). DEN induced UDS in hepatocytes of rats treated orally with 50 mg/kg b.w. (Mirsalis et al., 1982).

In germ cells of male mice (meiotic and post-meiotic stages upto mid-spermatids) treated i.p. with 4-8 mg DMN/kg b.w. or with 125 mg DEN/kg b.w., no induction of UDS was observed, indicating that no activated compound reached the testes (Sega, 1982; Racine and Schmid, 1983). Also in spermatocytes of rats after oral exposure and after inhalation, no induction of DNA repair by DMN could be detected (Doolittle et al., 1984; Working and Butterworth, 1984).

Cyclic nitrosamines

In vitro studies

In bacterial assays using the standard plate test with Salmonella typhimurium or Escherichia coli in the presence of rat liver S-9 for metabolic activation cyclic nitrosamines showed a weak mutagenic activity.

Smaller ring compounds were less mutagenic than larger ring compounds (McCann et al., 1975; Zeiger and Sheldon, 1978; Rao et al., 1979b, 1981a, 1982; Katz et al., 1980; Ashby, 1981; Araki et al., 1984; Phillipson and Ioannides, 1985). Liquid preincubation enhanced the mutagenic activity. The activity of the larger ring compounds was enhanced more in the liquid preincubation assay than the activity of the smaller ring compounds (Rao et al., 1979b; Araki et al., 1984). At preincubation of S. typhimurium with NMOR or NPYR and liver S-9 in a weakly acidic medium, a higher mutagenic activity was found than at preincubation in a neutral medium (Negishi and Hayatsu, 1980; Guttenplan, 1984). Marked species differences in bioactivation were seen. The hamster in contrast to the rat was a very effective activator (Weinstein et al., 1981; McCoy et al., 1982; Araki et al., 1984; Phillipson and Ioannides, 1985). Hepatocyte-mediated metabolic activation appeared to be more effective than activation by liver homogenate fractions (Ashby, 1981; Raineri et al., 1981; Rumruen and Pool, 1984). Also human liver homogenate fractions activated NPIP, NMOR and NPYR to mutagenic substances in assays with S. typhimurium (Bartsch et al., 1976; Sabadie et al., 1980; Phillipson and Ioannides, 1985). The mutagenic potency of cyclic nitrosamines found in Salmonella assays and the carcinogenic potency of these compounds in animal studies showed a direct relationship (Andrews et al., 1978; Rao et al., 1979b).

In S. cerevisiae NPIP induced gene-mutations in the presence of a metabolic activation system (Larimer et al., 1978). NMOR induced forward mutations in S. pombe P1 and mitotic aneuploidy in S. cerevisiae D6, but no mitotic recombinations were found in S. cerevisiae D1, D7, D4, T1 and T2 (Ashby, 1981). In a growth-mediated assay with Aspergillus nidulans NM showed mutagenic activity (Bignami et al., 1981).

In the higher plant Arabidopsis thaliana NM did not induce mutations (Veleminsky and Gichner, 1968).

Gene-mutations in mammalian cells in vitro were induced by NPIP, NMOR and NPYR in the presence of rat liver microsomes or primary rat hepatocytes (Bartsch et al., 1980; Ashby, 1981; Jones and Huberman, 1980; Ho et al., 1984).

In baby hamster kidney cells, NMOR induced gene-mutations and chromosomal aberrations. No chromosomal aberrations were detected in an epithelial-like cell line from rat liver (possessing metabolizing capacity) (Ashby, 1981).

A weak induction of SCE's in Chinese hamster ovary cells in vitro was

caused by NMOR at a dose of 1000 µg/ml in the presence of a metabolic activation system (Ashby, 1981). In human leucocytes in vitro NPIP induced a dose-dependent (2x) increase of SCE's in the presence of rat liver S-9 (Ho et al., 1984).

No tests for SCE induction in vitro with NPYR were available.

In a cell transformation assay with BHK-21 cells NMOR showed a positive result at concentrations ≥ 2.5 µg/ml (= LD50) in the presence of a metabolic activation system (Ashby, 1981).

Studies in Drosophila

NPIP induced sex-linked recessive lethals, but no chromosome loss in Drosophila melanogaster males (Nix et al., 1979; Zimmering, 1982). However Zimmering (1982) observed chromosome loss after treatment of (ring-X) males with low toxic doses of NPIP (LD4) followed by mating with repair-proficient females. Mating with repair deficient females enhanced the effect.

In D. melanogaster NMOR induced sex-linked recessive lethal mutations and translocations (IARC, 1978; Nix et al., 1980).

NPYR induced also sex-linked recessive lethals in D. melanogaster (Nix et al., 1980).

In the sex-linked recessive lethal test, NPIP showed the highest activity, followed by NMOR and NPYR (Nix et al., 1979, 1980).

In tests with Drosophila for somatic mutations and recombinations with both NM and NPIP, Würgler and Vogel (1985) reported positive results.

In vivo mammalian studies

No dominant lethal mutations in rats were induced by i.p. doses of 50 or 100 mg NMOR/kg b.w. (IARC, 1978).

Chromosomal aberrations in lymphocytes of rats were induced by NMOR at i.p. doses ≥ 250 mg/kg b.w. (Newton et al., 1977).

In micronucleus tests in bone-marrow of mice, a positive and a negative result were found with NMOR. In the positive assay an i.p. dose of 80% of the LD50 was used while in the negative assay 50% of the LD50 was the highest dose (LD50 i.p. mouse 64 mg/kg b.w.) (Ashby, 1981).

SCE's in bone-marrow of mice were induced by NPIP, NMOR and NPYR at i.p. doses ≥ 21 , ≥ 37.5 and ≥ 25 mg/kg b.w., respectively (Parodi et al., 1983).

SCE induction in peripheral blood lymphocytes of mice, treated i.p. at

doses ≥ 75 mg NMOR/kg b.w., was observed by Kligerman et al. (1985)

Host-mediated assays

In an i.p. host-mediated assay in mice, with Salmonella typhimurium as indicator organism, NMOR showed mutagenic activity at an oral or i.m. dose of 100 mg/kg b.w. (IARC, 1978).

In an intrasanguineous host-mediated assay with the same indicator organism, 0.2 mg/kg b.w. NMOR, given orally, produced already a significant, mutagenic activity in the liver (Edwards et al., 1979b).

In an intrasanguineous host-mediated assay in rats, with S. cerevisiae indicator organism, NPIP, injected s.c., showed a positive response in lung and liver (highest activity in lung) (Fahrig and Remmer, 1983).

When the indicator organism was injected intratesticularly, NPIP (given s.c.) did not reveal any mutagenic activity in testes (Fahrig and Remmer, 1983).

Assays for DNA damage

In vitro studies

In tests for detecting indirect DNA damage, measuring differential survival between DNA-repair proficient and DNA-repair deficient strains of bacteria, NPIP showed positive and negative results in Bacillus subtilis and Escherichia coli, both in the presence of a metabolic activation system (Ashby, 1981; Suter and Jaeger, 1981).

NMOR showed a weak effect in B. subtilis without metabolic activation; with activation a negative result was found (Ashby, 1981). Negative results were found for NMOR in a differential killing test with E. coli and Saccharomyces cerevisiae both in the absence and the presence of a metabolic activation system (Ashby, 1981).

In a prophage induction assay with E. coli in the presence of hamster liver S-9 NPIP and NPYR showed a DNA damaging effect. NMOR revealed borderline activity in this assay (Elespuru, 1984).

Single strand breaks in DNA of primary rat hepatocyte cultures were induced by NPIP (Bradley and Dysart, 1981; Sina et al., 1983). No induction of double strand breaks was detected (Bradley and Dysart, 1981).

Induction of UDS by NPIP in a culture of rat tracheal epithelium, was found by Ide et al. (1981).

NMOR induced UDS in human fibroblasts in vitro but not in human HeLa cells

both in the presence of a metabolic activation system (Ashby, 1981). NMOR and NPYR induced UDS in primary cultures of rat hepatocytes (Williams and Laspia, 1979; Probst et al., 1981; McQueen et al., 1983).

In vivo mammalian studies

NMOR induced inhibition of DNA synthesis in liver epithelium and renal tubular epithelium of mice, treated i.p. (Amlacher, 1982).

NPIP, NMOR and NPYR inhibited DNA synthesis in oesophageal epithelium of rats, treated i.p. NPIP and NM showed similar activity (50% inhibition at an i.p. dose of 1.9 and 2.5 mg/kg b.w., respectively), while NPYR was less active (50% inhibition at an i.p. dose of 44 mg/kg b.w.) (Mirvish et al., 1978). NPIP also inhibited DNA synthesis in tracheal and oesophageal epithelium of Syrian hamsters (50% inhibition at an i.p. dose of 59 and 114 mg/kg b.w., respectively) (Mirvish et al., 1978).

Induction of single strand breaks in liver DNA of mice and/or rats, treated i.p. with NPIP, NMOR or NPYR, was seen (Stewart and Farber, 1973; Schwarz et al., 1979; Brambilla et al., 1981; Stewart, 1981). NMOR induced both single and double strand breaks in liver DNA of rats, treated i.p. (Stewart and Farber, 1973). Repair of single strand breaks was incomplete after 14 days, while double strand breaks were repaired after 6 days (Stewart and Farber, 1973; Stewart, 1981). Stewart and Farber (1973) did not observe any induction of double strand breaks by NPIP; NPYR was not examined for induction of double strand breaks.

N-Nitrosothiazolidine

In a bacterial assay with Salmonella typhimurium nitrosothiazolidine induced mutagenic activity without metabolic activation with a clear dose-response relationship; metabolic activation decreased the mutagenic activity (Mihara and Shibamoto, 1980; Sekizawa and Shibamoto, 1980; Umano et al., 1984).

However Fiddler et al. (1984) did not detect any mutagenic activity of nitrosothiazolidine in S. typhimurium, but another method of synthesis for nitrosothiazolidine was used.

Nitrosothiazolidine was not tested for mutagenic activity in mammalian cells in vitro or in vivo.

Indirect DNA-damage was induced by nitrosothiazolidine in a "rec-assay" with Bacillus subtilis without a metabolic activation system (Loury et al., 1984).

UDS was induced by nitrosothiazolidine in primary rat hepatocyte cultures (Loury et al., 1984).

In vivo studies for effects on DNA are not available.

Nitrosodiethanolamine

In bacterial assays with various Salmonella typhimurium strains, using the plate test, without or with liquid preincubation, in the absence or the presence of a metabolic activation system derived from rats or hamsters, NDELA did not show mutagenic activity at concentrations up to 2 mg/plate (Rao et al., 1979a; Andrews and Lijinsky, 1980; Gilbert et al., 1981; Lee and Guttenplan, 1981; Lijinsky and Andrews, 1983). A weak mutagenic effect was found by Andrews and Lijinsky (1980) but only at high concentrations (5 mg/plate) in the presence of a metabolic activation system. Prival et al., (1982) found a mutagenic effect in bacterial systems at high concentrations (5-15 mg/plate) in a plate-incorporation assay in the presence of hamster liver S-9 for metabolic activation. No activity was found when rat liver S-9 was used for activation. Eisenbrand et al., (1983) did not find a clear mutagenic effect of NDELA in S. typhimurium in the absence and the presence of rat hepatocytes for metabolic activation (concentrations 0.3-6.7 mg/plate). However incubation with alcoholdehydrogenase in the presence of NAD, converted NDELA to a potent mutagenic substance.

Hesbert et al. (1979) found a weak, mutagenic activity in S. typhimurium at concentrations of 1-10 µl/plate without metabolic activation.

A weak induction of mitotic gene conversions by NDELA in Saccharomyces cerevisiae D7-144, without exogenous metabolic activation, was observed, when the cells were incubated in an acidic medium. In neutral medium the mutagenic activity was suppressed (Mehta and Von Borstel, 1984).

In Drosophila melanogaster NDELA caused a weak induction of sex-linked recessive lethal mutations at doses varying from 0.01-2.0 mM applied topically on late embryonic and early larval stages. The same authors also found a significant induction of somatic mutations (Fahmy and Fahmy, 1984). No assays for mutagenic activity in mammalian cells in vitro were available.

No induction of chromosomal aberrations or micronuclei in bone-marrow cells of mice, treated i.p. with NDELA (2 or 10 g/kg b.w.) was observed by Gilbert et al. (1981).

In an intrasanguineous host-mediated assay in mice with Escherichia coli as indicator organism, NDELA showed a mutagenic response, but the response was very much less than with DMN or DEN (Kerklaan et al., 1981).

In a prophage induction assay with E. coli in the presence of hamster liver S-9, NDELA did not reveal a DNA damaging effect (Elespuru, 1984).

No assays for effects on DNA in mammalian cells in vitro or in vivo were available.

Tobacco specific nitrosamines (TSNA)

In bacterial assays with Salmonella typhimurium in the presence of a metabolic activation system, NNN and NNK induced mutagenic activity in strains detecting base-pair substitution (Andrews et al., 1978; Bartsch et al., 1980; Hecht et al., 1983a). NAB, NAT and NNA were not tested in bacterial assays.

No assays for a mutagenic effect of the afore mentioned TSNA in non-mammalian eukaryotic systems or in mammalian systems in vitro or in vivo were available.

NNN and NNK induced UDS in cultured primary rat hepatocytes (Williams and Laspia, 1979).

NNN inhibited DNA synthesis in rat oesophageal epithelium after i.p. treatment with doses ≥ 5 mg/kg b.w. (Mirvish et al., 1978).

2.4 Observations in man

2.4.1 Case-studies

In early literature a few cases of industrial poisoning due to inhalation of dimethylnitrosamine were described. Exposure levels were not given. Freund (1937) described two cases of poisoning. In one case death occurred. Autopsy revealed centrilobular necrosis of liver cells and signs of intensive regenerative proliferation. Barnes and Magee (1954) and Jacobson et al. (1955) reported additional cases of non-lethal dimethylnitrosamine poisoning with liver damage and elevated body temperature. Liver cirrhosis was diagnosed in one out of two cases after a 10 months chronic poisoning (Barnes and Magee, 1954).

A case of fatal criminal poisoning was reported (Fleig et al., 1982; Pedal et al., 1982). The victim had been fed repeated doses of dimethylnitrosamine (total dose estimated to be 20-25 mg/kg b.w.) over a period of many months. The patient's disease was dominated by a chronic degenerative liver failure. Pathology after a 32 months long clinical course, revealed micronuclear cirrhosis and bile duct proliferation.

Cooper and Kimbrough (1980) reported nausea, vomiting and abdominal pain in 5 human beings within a few hours after consuming lemonade containing dimethylnitrosamine (concentration unknown). Laboratory tests showed abnormalities confined to the liver and platelets. Two of the five victims died. Autopsy showed necrotic hepatocytes throughout the liver lobules. Cooper and Kimbrough (1980) reported that one of the three survivors (age 2.5 years) showed continuous hepatosplenomegaly and elevated liver enzymes in addition to chronic active hepatitis, indicative for possible extreme and permanent liver damage. Herron and Shank (1980) showed that DNA in liver of one of the victims that died, was methylated at N7 and O6 position of guanine.

In general the main characteristic of human dimethylnitrosamine intoxication is liver damage with the clinical symptoms of ascites, elevated temperature and in cases of severe intoxication, hemorrhagic diathesis. A lethal dose of dimethylnitrosamine produces acute centrilobular dystrophy of the liver. Should the individual survive for a longer period of time, scarring and incipient regenerative processes (i.e. subacute dystrophy) develop. Repeated administration of small doses leads to micronuclear cirrhosis. Centrilobular pattern in these protracted cases is no longer recognizable (Pedal et al., 1982). None of the cases described in the literature developed hepatic malignancy (Fleig et al., 1982).

2.4.2 Epidemiology

Nitrosamines found in man's environment, may be involved in the causation of cancer. However, a clear cause/effect relationship between exposure and the incidence of certain cancers in man could not be demonstrated, nor was the exposure quantified. Also the lack of a truly unexposed population made evaluation of the epidemiological studies difficult.

In addition individual exposure to endogenous nitrosamines is affected by dietary components. Low levels of exposure to nitrosamines may be sufficient to cause cancer in cases where dietary deficiencies (vitamins,

trace elements, antioxidants) or excesses (high salt diet) are present. Data from a number of epidemiological studies are only suggestive (see below) and indicate that further investigations must be performed (as cited in NAS, 1981; Bartsch and Montesano, 1984; O'Neill et al., 1984; Preussmann, 1984).

The well-established correlation between exposure to tobacco smoke and risk of cancer of the upper respiratory tract strongly suggests that also tobacco specific nitrosamines contribute to the induction of these malignancies (Bartsch and Montesano, 1984).

Patients with chronic atrophic gastritis, pernicious anemia or those who have undergone gastrectomy, can be at increased risk for stomach cancer. The achlorhydric stomachs of these patients may provide a suitable environment for the intragastric formation of carcinogenic nitroso compounds (Charnley, et al., 1982; Mirvish, 1983). Endogenous nitrosation does occur in the human stomach (Ohshima and Bartsch, 1981, Groenen et al., 1984) but relation to the induction of upper gastro-intestinal cancer remains to be proven (Bartsch and Montesano, 1984).

Extensive research in the People's Republic of China has suggested that nitroso compounds and/or their precursors may be among the etiological factors involved in the high incidences of oesophageal cancer in certain provinces in Northern China (Li et al., 1980). The suggestion was supported by the discovery of pharyngeal and oesophageal cancers in chickens in the same environments and with the same dietary sources as the population in the high-risk areas (Li et al., 1980). Yang et al. (1982) concluded; although nitrosamines are the most probable carcinogens for oesophageal cancer in Northern China, there is not enough evidence to firmly establish this. Lu et al. (1984) reported that inhabitants living in a high-risk area for oesophageal cancer in Northern China excreted significantly more nitrate and nitrosamino acids than those in a low-risk area. When the inhabitants of the high-risk area were given vitamin C, the level of urinary nitrosamino acids was reduced to that found in the low-risk area.

Hong-Kong was defined as an unusually high incidence area for nasopharyngeal carcinoma, primary liver cancer and cancer of the oesophagus. The combination of contamination of food stuffs with carcinogens and nutritional deficiencies may play a role. An association between the high intake of salted fish and nasopharyngeal cancer and between zinc deficiency and oesophageal cancer, is suggested (Fong, 1982).

An association between endogenously produced nitrosamines and an increased risk for bladder cancer in bilharzia infested populations in North African countries is suggested. Nitrosamines can be produced because of increased nitrate to nitrite conversion and the acidic pH of the urine. No conclusive proof was provided by a study among a village population of young men in Egypt (Hicks, 1982).

A relation between the presumed exposure to nitroso compounds (in particular nitrosoureas) and their precursors and the development of cerebral tumours in man, has been suggested. Three case-control studies among intracranial tumour patients in California, USA, were performed. The studies showed various limitations (Preston-Martin and Henderson, 1984).

Helgason and Jonasson (1981) reported an increased incidence of juvenile diabetes mellitus among boys in the Icelandic population. An association with the consumption of large amounts of Icelandic smoked mutton by the parents at about the time of conception was suggested. The diabetic response was detected also in male offspring of mice fed Icelandic smoked mutton (Helgason et al., 1982). Nitrosothiazolidine (ppb's) and nitrosothiazolidine-4-carboxylic acid (ppm's) were detected in smoked meat products. Both nitrosamines produced an age-dependent diabetic response in mice after intraperitoneal injection (Helgason et al., 1984).

An exception to all the suggestive data are the studies among smokeless-tobacco chewers and snuff dippers in some southern states of America and in Scandinavia. In these studies an association between human cancer of the oral cavity and the use of smokeless-tobacco has been confirmed. No chemical carcinogens other than the tobacco specific nitrosamines were detected in smokeless-tobacco in relatively high concentrations (see Table VII). The evidence that the oral use of snuff (is not chewed but left stationary in the mouth) is a cause of cancer of the oral cavity, especially of the buccal mucosa and gums, is convincing (Winn, 1984; IARC, 1985). Case-control studies have shown that chronic users of oral snuff have an about 50x increased risk for cancer of the gum and buccal mucosa compared to controls and that the risk increases with the number of years of snuff dipping (as cited in Hoffmann et al., 1985). The evidence for the carcinogenicity of chews (with tobacco as only component) is limited. More research is needed to clarify the risk of oral cancer related to tobacco chewing in US and other populations. Examination of the effects of smoking, alcohol and diet, which may modify the risk of cancer from smokeless-tobacco, and de-

termination whether cessation of the habit is associated with a reduced risk of cancer, will be important (Winn, 1984; IARC, 1985). As yet little evidence exists regarding the effect of smokeless-tobacco on other sites than the oral cavity, although preliminary evidence suggests an increased risk for oesophageal cancer (Winn, 1984).

In India, population based incidence rates and relative frequencies of oral and pharyngeal cancer in six major cancer hospitals indicate that these forms of cancer occur frequently. Case-control studies revealed an association with tobacco chewing and bidi smoking. Chewing tobacco, used in this country, contained ppm's nitrosonornicotine and 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone (Bhide et al., 1984).

There is inadequate evidence that the nasal use of snuff is carcinogenic to man. No study was available that specifically addressed the possible carcinogenicity of nasal use of snuff formulated in Northern America or Western Europe (IARC, 1985).

2.5 Summary

Man may be exposed to preformed nitrosamines via ingestion, inhalation and dermal contact. In general nitrosamines occur in the environment in trace amounts. In addition nitrosamines may be formed endogenously from various nitrosatable compounds.

Nitrosamines require metabolic activation to reactive metabolites to develop their biologic effects. The generally accepted primary reaction is alpha-hydroxylation and subsequently dealkylation of the reactive intermediate to an alkyl diazohydroxide and finally the formation of an alkylating agent resulting in alkylation of cellular macromolecules. Besides the activation pathway, also deactivation pathways exist.

Most nitrosamines are carcinogenic in all animal species examined and induce tumours of different cell types and in different organs from one species to the next. The organotropism of the carcinogenic effect may affect a number of organs and organ systems, even though the liver obviously is the preferred target organ. The organotropism depends not only on the chemical structure of the molecule but also a.o. on the daily dose applied and on the strain within the same animal species. The route of application plays a minor role in the organotropism of the carcinogenic effect of nitrosamines. It is highly probable that most nitrosamines also act as a carcinogen to humans. Among the 14 nitrosamines reviewed in this document, nitrosothia-

zolidine, NAT and NNA were tested insufficiently to evaluate their carcinogenic activity.

In bacterial assays nitrosamines showed a weak mutagenic activity in the presence of a metabolic activation system. Distinct responses were observed under special conditions such as; a) liquid preincubation b) metabolic activation by hamster liver fractions instead of rat liver fractions c) metabolic activation by primary hepatocytes instead of S-9 liver mix and d) weakly acid medium instead of neutral medium.

Human liver fractions activated the cyclic nitrosamines NPIP, NMOR and NPYR in bacterial assays but did not activate the dialkyl nitrosamines DMN, DPN and DBN.

For the dialkyl nitrosamines an inverse relationship between mutagenic potency in the Salmonella assay and carcinogenic potency in animal studies was observed. For the cyclic nitrosamines a direct relationship was found. In all other in vitro systems (fungi, mammalian cells) nitrosamines showed mutagenic activity under conditions of metabolic activation. Also in these systems species differences in bioactivation were observed. For the induction of gene-mutations, the activity of the dialkyl nitrosamines decreased with increasing chain length. The chromosomal aberration types induced by DMN and DEN, were mainly chromatid exchanges whereas those induced by DPN and DBN were predominantly breaks.

Nitrosamines are efficient inducers of sex-linked recessive lethals in Drosophila.

In vivo studies with nitrosamines revealed mutagenic activity in somatic cells at relatively high doses (compared to LD50 values).

In germ cells no effects were observed.

Nitrosamines caused DNA damage in somatic cells in vitro and in vivo. Single strand breaks were induced by nitrosamines in DNA of somatic cells and NMOR induced double strand breaks, too.

In vivo studies with nitrosamines revealed inhibition of DNA synthesis in germ cells, but no DNA damage in germ cells was found.

N-Nitrosothiazolidine and the TSNA were tested insufficiently to evaluate their mutagenic activity.

Early findings of acute toxicity of DMN (liver cirrhosis and necrosis) in occupationally exposed workers, were confirmed in recent criminal poisoning cases with the same compound.

It is difficult or impossible to demonstrate a cause/effect relationship in

the general population between exposure to low levels of nitrosamines and the incidence of certain cancers due to the insensitivity of the epidemiologic instruments and to the lack of a truly unexposed population that could be used as controls. In addition individual exposure to endogenous nitrosamines is affected by dietary components. Data from a number of epidemiological studies are only suggestive.

An exception to the suggestive data are the studies among snuff (= smokeless-tobacco) dippers and smokeless-tobacco chewers. There is sufficient evidence that the oral use of snuff (called snuff dipping) of the types commonly used in some southern states of North America and in Scandinavia, is carcinogenic to humans. There is limited evidence that chewing smokeless-tobacco of the types commonly used in these areas, is carcinogenic to humans.

There is inadequate evidence that the nasal use of snuff is carcinogenic to humans.

3. ECOTOXICITY

This chapter is focused entirely on the toxicity of nitrate, nitrite and ammonia to single species of aquatic organisms.

The relatively few data available concerning the main item of this document: nitrate, are listed in the Tables XI to XIII. The numerous data concerning nitrite and ammonia have been reviewed to present a broad survey of the most important issues.

3.1 Nitrate

The toxicity of nitrate greatly depends on the cationic composition of the water.

In acute tests with fish (several species) and eggs of freshwater snails, potassium nitrate has been found to be 3 to 5 times more toxic than sodium nitrate, with the same concentration of nitrate ion in solution; for waterfleas this factor was 15 to 20 (Dowden and Bennett, 1965; Westin, 1974). Calcium nitrate has been reported to be about 65% less toxic compared with sodium nitrate for sticklebacks in an experiment of 10 days (Jones, 1939; cited by Westin, 1974). In a test with chinook salmon and rainbow trout fingerlings there was a small but significant influence of the salinity of the test solution, with higher lethal toxicity in seawater compared with freshwater (Westin, 1974).

All following toxicity data, including those in the corresponding tables, are expressed as NO_3^- mg/l.

3.1.1 Freshwater organisms (short-term)

In Table XI detailed information is given.

For sodium nitrate LC50 values are ranging from about 500 to 9000 mg/l, and for potassium nitrate from 24 to 3355 mg/l. With both test substances, the waterflea Daphnia magna was found to be the most sensitive species.

3.1.2 Freshwater organisms (long-term)

In Table XII detailed information is given.

Only data on long-term effects on fish were available.

Early life stages of salmonid fish are sensitive to low nitrate concentrations; two subspecies of Salmo gairdneri are the most sensitive ones, with NOLC values < 5 mg/l (eggs) and 5 mg/l (eggs, fry) for steelhead trout and rainbow trout, respectively.

The only other data available are from an experiment in which channel catfish (Ictalurus punctatus) and largemouth bass (Micropterus salmoides) simultaneously were exposed to rising nitrate levels up to 400 mg/l, due to their own nitrogen excretion. With exception of 30% mortality of the bass during the first 30 days of exposure, there was no mortality or noticeable effect on growth and feeding activity of both species.

3.1.3 Marine organisms (short- and long-term)

In Table XIII detailed information is given.

The growth (measured as chlorophyll-a content in the test-culture) of 10 different species of benthic diatoms (algae) was not affected by concentrations up to 418 mg/l (added as potassium nitrate), when exposed for about 7 days (long-term). At the highest test level of 1048 mg/l, the growth of 5 species was inhibited with 30% to 65%.

Acute toxicity tests with other non-fish species showed LC50 values of 32.5 - 98.4 mg/l (48-hr) for the worm Ophryotrocha diadema and $\geq$ 11500 mg/l (96-hr) for the bivalve molluscs Mercenaria mercenaria (hard clam) and Crassostrea virginica (American oyster), with nitric acid and sodium nitrate as test substance, respectively.

The 168-hr LC50 values for fingerlings of two salmonid fish species (Oncorhynchus tshawytscha and Salmo gairdneri) were 4000 mg/l.

For larvae of four marine fish species, 24-hr LC50 values were as high as 15000 - 22000 mg/l, indicating an aspecific effect due to the high osmolarity of the test solution on lethal toxicity.

3.1.4 Summary

The toxicity of nitrate greatly depends on the cationic composition of the water.

According to the proposal of Canton and Slooff (1979), nitrate can be classified as being very slightly toxic, with L(E)C50 values > 100 mg/l (short-term) and NOL(E)C values > 1 mg/l (long-term), with sodium nitrate in the water. This statement is valid for both freshwater and marine organisms.

With potassium nitrate and nitric acid as test substance, nitrate is slightly toxic ($10 < \text{LC50} < 100$ mg/l) to the freshwater organism Daphnia magna and the marine organism Ophryotrocha diadema, respectively, at short-term exposure. Based on the other data available, potassium nitrate

is very slightly toxic, too.

3.2 Nitrite

The toxicity of nitrite is highly dependent upon some environmental factors.

An increase in the pH with 2 units (within the range of 6 to 8.6) caused a decrease in the toxicity with a factor 5 to 8 in rainbow and steelhead trout fingerlings; at least for rainbow trout, this effect was most pronounced above pH 8 (Wedemeyer and Yasutake, 1978; Russo et al., 1981). In the bluegill even a factor 60 was found between pH 4 and 7 (Huey et al., 1982).

In both fish and other species chloride has been found to inhibit the sublethal and lethal toxicity, for example in steelhead trout fingerlings (Wedemeyer and Yasutake, 1978), coho salmon yearlings (Perrone and Meade, 1977), channel catfish (Tomasso et al., 1979, 1981), crayfish (crustaceans, Beitinger and Huey, 1981; Gutzmer and Tomasso, 1985), salamander larvae (Huey and Beitinger, 1980b) and common bullfrog larvae (Huey and Beitinger, 1980a). The protective effect of chloride can be influenced by the cationic composition and the pH. For rainbow trout fingerlings the protective effect of CaCl_2 was 18 times higher compared with NaCl at equal quantities of Cl^- ion (Wedemeyer and Yasutake, 1978). In contrast, there was little or no difference between the protective effect of NaCl and CaCl_2 in channel catfish (Tomasso et al., 1980).

Other minor factors that have been reported to influence the toxicity of nitrite are: total hardness (lower toxicity with a higher level of a mineral salt mixture; Wedemeyer and Yasutake, 1978), anions in solution (sulphate, nitrate, phosphate, Russo et al., 1981) and temperature (Huey et al., 1984). Some data concerning the influence of the developmental stage (weight) are contradictory: no significant difference in toxicity was found for rainbow trout ranging from 2 to 387 g (Russo et al., 1981; secondary source), while 10 g steelhead trout were somewhat more tolerant than those of 5 g (Wedemeyer and Yasutake, 1978); for fathead minnows a clear difference in tolerance was found, with smaller (0.3-0.8 g) fish significantly more tolerant than larger (0.9-3.3 g) fish. This difference was decreasing with time of exposure, but still existing after 96 hours (Palachek and Tomasso, 1984).

All following toxicity data are expressed as NO_2^- mg/l.

3.2.1 Freshwater organisms (short-term)

Non-fish species

Data on the short-term effects on algae were not available.

For bacteria, Pseudomonas putida, a 16-hr toxicity threshold (cell multiplication inhibition) of 82 mg/l has been reported; for several Protozoa (ciliates, flagellates) this value was 1.9-17.4 mg/l at exposures up to 72 hours (Bringmann, 1978; Bringmann and Kühn, 1977a, 1980; Bringmann et al., 1980).

For crustaceans, the 96-hr LC50 for the crayfish Procambarus clarkii is 28 mg/l without chloride and 75 mg/l with addition of 100 mg/l chloride (Gutzmer and Tomasso, 1985); (24-hr) EC50 (immobility) values for the waterflea Daphnia magna are 87-144 mg/l (Bringmann and Kühn, 1977b, 1982).

From all non-fish species tested, larvae of the salamander Ambystoma texanum were the most sensitive, with a 96-hr LC50 of 1.1 mg/l (pH 7, total hardness 140 mg/l, Cl⁻ 5 mg/l) (Huey and Beitinger, 1980b). Another amphibian, the bullfrog Rana catesbiana (larvae) seems less sensitive, with only sublethal methaemoglobinemia when exposed to up to 50 mg/l for 24 hours in similar water conditions (Huey and Beitinger, 1980a).

Fish

Based on the screened literature, salmonids are more sensitive to nitrite compared with other fish. For this reason the data for both groups of fish are presented separately.

Salmonids

Rainbow trout (Salmo gairdneri) appears to be the most sensitive species, with a 96-hr LC50 ranging from 0.56-1.78 mg/l (Cl⁻ 0.0-1.7 mg/l) or from 11.5-17.4 mg/l (Cl⁻ 10 mg/l), at pH between 7.5 and 8.6 in hard water (hardness about 200 mg/l CaCO₃). The lowest value found within the pH range 6.4-8.6 is 0.36 mg/l (Russo et al., 1981). For steelhead trout (S. gairdneri) 96-hr LC50 values range between 1.0 mg/l (5 g fingerlings, pH 6, hardness 25 mg/l CaCO₃, Cl⁻ 1.9 mg/l) and 102.6 mg/l (15 g fingerlings, hardness 25 mg/l CaCO₃, Cl⁻ 128 mg/l) (Wedemeyer and Yasutake, 1978). Values for chinook salmon (Oncorhynchus tshawytscha) and coho salmon (O. kisutch) are similar compared with steelhead trout (Westin, 1974; Perrone and Meade, 1977).

Non-salmonids

For channel catfish, Ictalurus punctatus (96-hr), roach, Leuciscus idus (48-hr) and fathead minnow, Pimephales promelas (96-hr) LC50 values of 23 and 42 mg/l (Colt and Tschobanoglous, 1976; Palachek and Tomasso, 1984b), 241 and 379 mg/l (Juhnke and Lüdemann, 1978), and 145 or 230 mg/l (2 size-classes, Palachek and Tomasso, 1984a), have been reported, respectively. Extremely different values were found for the bluegill Lepomis macrochirus: 48-hr LC50 values of 4.4 - 4.6 mg/l at pH 4 and 211 - 282 mg/l at pH 7.2 (Huey et al., 1982). The highest 96-hr LC50 values have been found for largemouth bass, Micropterus salmoides (460 mg/l; threshold) and for smallmouth bass, Micropterus dolomieni, and green sunfish, Lepomis cyanellus (both about 525 mg/l) at pH about 8 (Palachek and Tomasso, 1948b; Tomasso, 1986).

3.2.2 Freshwater organisms (long-term)

Non-fish species

The 8-d toxicity threshold values (cell multiplication inhibition) for green and blue-green algae are 235 and 824 mg/l, respectively (Bringmann and Kühn, 1978).

Data on crustaceans were not available.

Fish

For 1 g cutthroat trout (Salmo clarki) fry an asymptotic 36-d LC50 of 1.23 mg/l has been reported (sodium nitrite added, pH 7.8 - 8.0, hardness 199 mg/l as CaCO₃, Cl⁻ 0.44 mg/l). The maximum concentration with no mortality during this period was 0.66 mg/l (Thurston et al., 1978). Chronic exposure of juvenile steelhead trout (S. gairdneri) to 0.1 mg/l for 6 months in freshwater (pH 6.2, hardness 25 mg/l as CaCO₃, Cl⁻ 2.3 mg/l) caused no lethality, growth reduction, gill histological changes or hematological dyscrasias after 7 weeks of exposure. A concentration of 0.05 mg/l caused a significant increase in the methaemoglobin level (from 1.5% to 2.7% of total HB), but this slight effect probably has no biological significance (Wedemeyer and Yasutake, 1978).

In a 31-d growth trial with juvenile channel catfish, Ictalurus punctatus, in well water (pH 8.2, Cl⁻ 22 mg/l), a nitrite level of 8.6 mg/l caused no mortality. The NOEC (growth) was found to be 4.1 mg/l. No gill damage was apparent at any test level (up to 15.7 mg/l) (Colt et al, 1981).

3.2.3 Marine organisms (short- and long-term)

The growth of 10 different species of estuarine benthic diatoms (algae) was not affected by a concentration of 46 mg/l, when exposed for 7 days; a concentration of 460 mg/l caused a growth inhibition of about 10% to 100% (Admiraal, 1977).

For crustaceans, a 96-hr LC50 of 6 mg/l was found for the crayfish Procambarus simulans at low chloride ($\leq 5 \text{ mg.l Cl}^{-1}$) content of the water, and 28 mg/l for larvae of the giant Malaysian prawn Macrobrachium rosenbergii in 12 promille seawater (Beitinger and Huey, 1981; Armstrong et al., 1976). Least sensitive of the non-fish organisms were 2 species of bivalve molluscs in seawater, with 96-hr LC50 values of 3800 (hard clam, Mercenaria mercenaria) and 2400 (American oyster, Crassostrea virginica) mg/l (Epifanio and Srna, 1975).

For 107-183 days old larvae of 5 marine fish species, 24-hr LC50 values of 4047 to 8027 mg/l were found in seawater (34-36 promille salinity, pH 7.8) (Brownell, 1980).

Data on chronic effects on crustaceans and fish were not available.

3.2.4 Other data

As in mammals, the most common effect of nitrite in fish is methaemoglobinemia. Because of 1) the tolerance to high methaemoglobin levels (for example: salmonids up to 89%; channel catfish up to 100%), 2) the lack of correspondence between these levels and the physiological state as found in several studies, 3) the bioaccumulation of nitrite in blood and tissues, and 4) biochemical and structural changes in some tissues, it seems unlikely that hypoxia alone is causing (acute) death (Perrone and Meade, 1977; Tomasso et al., 1979; Margiocco et al., 1983; Arillo et al., 1984).

Nitrite also induced methaemoglobinemia in the amphibian Rana catesbiana (Huey and Beitinger, 1980).

Muscle (ether) extracts of rainbow trout, Salmo gairdneri, exposed to nitrite enriched water (1.48 mg/l, corresponding to the 48-hr LC50) for 12 to 48 hours showed mutagenic and DNA-damaging activity in Salmonella typhimurium and Escherichia coli, respectively, apparently due to the presence of nitroso-derivates (-amides). On the basis of the genetic mechanism and the influence of metabolic stimulation, these compounds were most probably formed in vivo (De Flora and Arillo, 1983).

3.2.5 Summary

The toxicity of nitrite is highly dependent on some environmental factors. Relatively high pH and Cl^- content (especially at high Ca^{++}) both have a protective effect.

According to the proposal of Canton and Slooff (1979), nitrite is very toxic ($\text{LC}_{50} < 1 \text{ mg/l}$) to freshwater organisms, in water with a low chloride content, at short-term exposure. On the basis of long-term exposure data, nitrite appears to be slightly toxic to freshwater fish, with an NOEC of 0.1-1 mg/l.

On the basis of short-term toxicity data, nitrite is moderately toxic ($1 < \text{LC}_{50} < 10 \text{ mg/l}$) to marine organisms. At long-term exposure, nitrite is very slightly toxic ($\text{NOEC} > 1 \text{ mg/l}$) to marine algae.

3.3 Ammonia

At least to fish, the toxicity of environmental "total ammonia" ($\text{NH}_3 + \text{NH}_4^+$) is greatly determined by the concentration of "un-ionized ammonia" (NH_3), due to the much greater permeability of gills for NH_3 compared with NH_4^+ , "ionized ammonia" (Alabaster and Lloyd, 1980; Hillaby and Randall, 1979; Thurston et al., 1981a).

There are several environmental factors that affect the toxicity of ammonia (Alabaster and Lloyd, 1980; Thurston et al., 1981a,b). The major factors are pH and temperature, both increasing the toxicity of (total) ammonia at higher levels, by increasing the concentration of "un-ionized ammonia". The most important of these two factors is the pH: an increase in pH value of 0.3 or 1.0 units will cause an increase in the NH_3 concentration with a factor of about 2 or 10, respectively. A rise in temperature of 10°C doubles the concentration of NH_3 (at least, above 10°C).

All following toxicity data are expressed as NH_3 mg/l.

3.3.1 Freshwater organisms (short-term)

Non-fish species

For snails (molluscs), LC_{50} values are 173 mg/l for Lymnaea sp. (eggs, 48-hr) and 90 (soft water) and 134 (hard water) mg/l for Physa heterostropha, respectively (Shell, 1983).

The 96-hr LC_{50} for the crustacean Daphnia pulicaria (waterflea) and Orconectes nais (crayfish) are 1.2 and 3.8 mg/l, respectively (Graeve et al., 1980; Hazel et al., 1982). Other crustaceans, the copepods Cyclops

vernalis and Mesocyclops leukarti are less sensitive, with a toxicity threshold (immobilization) of about 80 mg/l (Shell, 1983).

For the insect Stenelmis sexlineata Sand. a 96-hr LC50 of 8 mg/l has been reported (Hazel et al., 1982).

Data on the effects on algae were not available.

Fish

The toxicity of ammonia has been reviewed by the Working Party on Water Quality Criteria for European Freshwater Fish, a section of the European Inland Fisheries Advisory Commission (EIFAC, 1970). An updating of this review was published by Alabaster and Lloyd (1980). Another review is published by Haywood (1983).

Although the toxicity of ammonia greatly depends on the concentration of "un-ionized ammonia" (see 5.2.3), once entered the fish, either the "ionized" form or the "total ammonia" load appears to be toxic to fish (Hillaby and Randall, 1979).

Beside the main factors (pH, temperature) there are other factors that have been reported to affect the toxicity of ammonia. These factors are: dissolved oxygen concentration (a reduction increases the toxicity), alkalinity (bicarbonate concentration) in conjunction with free carbon dioxide level (effect on pH), salinity (decreasing toxicity with increasing salinity), acclimation to (sublethal) ammonia concentrations, developmental stage and sex, swimming speed and physical handling before testing.

Most (threshold) LC50 values for a variety of fish species are in the range of 0.2 to 2.0 mg/l (Alabaster and Lloyd, 1980). Salmonids are somewhat more sensitive than other fish, especially for very short periods of exposure. For example, Ball (1967) showed that for rainbow trout (Salmo gairdneri) the threshold LC50 of 0.50 mg/l was reached within one day, while similar values (range 0.35 to 0.50 mg/l) were found for four species of coarse (Cyprinid) fish (bream, Abramis brama; perch, Perca fluviatilis; roach, Rutilus rutilus; rudd, Scardinius erythrophthalmus), when exposure was extended to 2-4 days.

In general, for salmonid fish (threshold) LC50 values range from 0.15-0.50 mg/l, with fry and smolts being the most sensitive stages. Extremely low values have been reported by Rice and Stokes (1975) and Rice and Bailey (1980): 24-hr LC50 values of 0.07 and 0.1 mg/l for 85 days old fry and adults of rainbow trout, respectively, and a 96-hr LC50 value of 0.083 mg/l

for late alevins of the pink salmon, Oncorhynchus gorbuscha; these very low values were possibly caused by the use of tris-buffer and/or very soft water.

For most other fish values range between 0.6 mg/l (perch) and 2.0 mg/l (tench, Tinca tinca; carp, Cyprinus carpio).

A global overview of data published from 1979 to 1985 for both salmonids and other fish species showed similar results (references not listed).

The lowest sublethal concentration has been published for trout, S. gairdneri: a 48 hours exposition to 0.024 mg/l induced a significant alteration in various biochemical parameters (Ariello et al., 1981).

3.3.2 Freshwater organisms (long-term)

Non-fish species

For diatoms (algae) of the species Navicula seminulum, 5-d EC50 (growth) values of 320 (28 °C) and 420 (22 °C) mg/l were reported (Shell, 1983).

Data on long-term effects on crustaceans were not available.

Salmonid fish

For rainbow trout (Salmo gairdneri) exposed from egg through to fry stage, a 72-day LC50 of 0.056 mg/l is reported (Calamari et al., 1981). For rainbow trout fingerlings an 90-days NOEC of 0.2 mg/l was found (Daoust and Ferguson, 1984). In a 5-years study with rainbow trout (exposure times F0, F1 and F2: 11 months, 4 years and 5 months, respectively) concentrations up to 0.074 mg/l did not affect mortality, growth or number and viability of eggs, although the F1 fish did not spawn voluntarily at either 3 or 4 years of age. Histopathological lesions (gills, kidneys) were common in parental and F1 fish at 0.04 and in F2 fish at 0.02 mg/l (Thurston et al., 1984).

Some other data: 36-day and 62-day LC50 values: 0.56 (cutthroat trout, S. clarki; eggs) and 0.11 mg/l (sockeye salmon, Oncorhynchus nerka; eggs), respectively (Spehar et al., 1979, 1981). There was no mortality of chinook salmon (O. tshawytscha) fingerlings exposed for 6 weeks to 0.018 mg/l, but some hyperplasia of the gill epithelium was observed (Alabaster and Lloyd, 1980).

Fry of O. gorbuscha, pink salmon, exposed to 0.0024 mg/l from the alevin stage for 40 or 61 days, showed a significantly reduced length and weight; the NOEC was 0.0012 mg/l (Rice and Bailey, 1980).

Other data reviewed by Haywood (1983) indicate sublethal chronic effects (gill changes, distress) at levels of 0.003 to 0.008 mg/l for salmonids.

Non-salmonid fish

Exposure of eel (Anguilla japonica) for 9 weeks to more than 0.08 mg/l reduced the growth significantly; gill changes (histological, pathological) were observed at concentrations $\geq$ 0.15 mg/l. The NOEC (growth) was estimated to be 0.04 mg/l (Yamagata and Niwa, 1982; Jap., English abstract). For the European eel (A. anguilla) the NOEC (growth, 6 weeks exposure) was 0.15 mg/l (Sadler, 1981).

For green sunfish, Lepomis cyanellus, exposed from the blastula stage up to 40 days post-hatching, an NOEC (growth) of 0.33 mg/l was estimated (McCormick, 1984).

3.3.3 Marine organisms (short- and long-term)

Non-fish species

The growth of ten different species of estuarine benthic diatoms (algae) was inhibited at a total ammonia level of 18 mg/l (12 °C, pH 8), corresponding with approximately 0.4 mg/l NH₃, at long-term exposure (7 days). The NOEC was 9 mg/l total ammonia, corresponding with approximately 0.2 mg/l NH₃ (Admiraal, 1977).

From two bivalve molluscs (Mercenaria mercenaria, the hard clam and Crassostrea virginica, the American oyster), the clam was the most sensitive with a 96-hr LC₅₀ of 590 mg/l NH₄Cl (20 °C, pH 7.70 - 7.96), corresponding with NH₃ concentrations between 2.5 and 7.5 mg/l (Epifanio and Srna, 1975).

Lower amounts (0.044 and 2.46 mg/l, depending on test medium used) inhibited the growth of another mollusc, the snail Biomphalaria glabrata (juveniles), in experiments up to 9 days; the NOEC values in most experiments were 0.017 and 0.98 mg/l (Thomas et al., 1976).

For the copepod Nitocra spinipes (crustaceans) the 96-hr LC₅₀ is between 1 and 2.5 mg/l (Lindén et al., 1979).

Fish

For larvae of five fish species, 24-hr LC₅₀ and EC₅₀ (first-feeding incidence) values ranged from 0.44-0.56 mg/l and 0.12-0.27 mg/l, respectively (Brownell, 1980).

In two other species growth ceased at levels of 0.46-0.94 mg/l (Dover sole) or 0.37-1.09 mg/l (turbot), at lower and higher pH values respectively. The growth of these species was not affected at concentrations of 0.05-0.08 mg/l (soles) and 0.10-0.13 mg/l (turbot) (Haywood, 1983).

In a long-term test with smallmouth bass (Micropterus dolomieu), exposed from the late embryo stage for 32 days, NOEC (growth) values of 0.044 (pH 6.6) and 0.612 (pH 8.7) were calculated (Broderius et al., 1985).

3.3.4 Summary

The toxicity of total ammonia ($\text{NH}_3 + \text{NH}_4^+$) greatly depends on the quantity of "un-ionized ammonia" (NH_3) in the water. This quantity and consequently the toxicity of environmental ammonia is enhanced by an increase in pH and temperature.

According to the proposal of Canton and Slooff (1979), NH_3 can be classified as being very toxic ($\text{LC}_{50} < 1 \text{ mg/l}$) for freshwater organisms, at short-term exposure. At long-term exposure, NH_3 is very toxic ($\text{NOEC} < 0.01 \text{ mg/l}$) to freshwater organisms, too.

For marine organisms, NH_3 is very toxic at short-term exposure and moderately toxic ($0.01 < \text{NOEC} < 0.1 \text{ mg/l}$) at long-term exposure.

4. RISK EVALUATION

4.1 Human toxicity

Experimental studies showed that intake of nitrate by the oral route can result in an increased methaemoglobin blood level in healthy young infants as well as in healthy adult animals. Data on clinical cases of methaemoglobinemia in infants cannot be evaluated in relation to nitrate intake, because other factors, like bacterial contamination of drinking water and gastrointestinal infection in infants are often also involved. No data on subchronic and chronic ingestion of nitrate in man are known except for the epidemiological studies with concern to the occurrence of tumours. For this reason a no-toxic effect level of 500 mg sodium nitrate/kg b.w. could have derived from a chronic feeding study with rats, in which at the next higher dose level of 2500 mg sodium nitrate/kg b.w. dose level a slight growth inhibition was noticed. However, as the chemobiokinetics and metabolism of rats differ from that of man, the rat has to be considered as a less suitable animal species to study the toxicity of nitrate. In a subacute study with rabbits effects were observed, but this study was considered less relevant for risk evaluation because nitrate was administered as a pulse dose by means of gelatin capsules, whereas humans have their daily oral intake of relative low quantities of nitrate in food commodities and drinking water. As the toxicity of nitrate is mainly determined by the amount of nitrate converted to nitrite, the no-toxic effect level of nitrate should therefore rather be calculated from the conversion rate of nitrate to nitrite in man and the no-toxic effect level of nitrite -see below.

Nitrate is not mutagenic in bacteria and mammalian cells in vitro. In in vivo experiments chromosome aberrations were induced in rat bone marrow, but it cannot be excluded that formation of N-nitroso compounds was responsible for the chromosome damage. It was concluded from animal studies that nitrate itself had no carcinogenic properties.

Children up to 3 months of age have to be recognized as a risk group. They may ingest daily relatively high amounts of nitrate via drinking water and they are more vulnerable for adverse effects of nitrate than adults because relatively more nitrate is reduced to nitrite and the methaemoglobinemia is induced at a lower dose level.

No adequate data on the subchronic or chronic intake of nitrite by man are available, therefore the no-toxic effect level of 10 mg sodium nitrite/kg b.w. or 6.7 mg NO_2^- /kg b.w. was derived from a long-term toxicity study with nitrite administered via the drinking water to rats. In this study, the most sensitive parameter, the formation of methaemoglobin, was observed from the next lowest dose level of 100 mg sodium nitrite/kg b.w. onwards. Nitrite is mutagenic and causes morphological transformation in in vitro systems. Mutagenic activity was also found in a combined in vivo-in vitro experiment with Syrian hamsters. In vivo experiments led to conflicting results. The mechanism in some in vitro systems probably is due to deamination, while in the in vivo experiments endogenous formation of N-nitroso compounds is likely to play a role.

Although a few studies with nitrite revealed some slight increases in tumour incidences, which possibly resulted from the nitrosamines present in the diet fed to mice or rats, it is the final conclusion that there is no distinct evidence that nitrite itself is carcinogenic.

With the realistic assumption that in adults 5% nitrate is converted to nitrite, the no-toxic effect level for nitrate can be derived from that of nitrite in rats: $100\%/5\% \times 10 \text{ mg/kg b.w.} = 200 \text{ mg sodium nitrate/kg b.w.}$ or 146 mg NO_3^- /kg b.w. The average daily intake of adults is estimated to be 110 mg NO_3^- /day or 1.83 mg NO_3^- /kg b.w./day, so there is a margin of safety of a factor 80 between the actual intake and the no-effect level.

The bacterial reduction of nitrate to nitrite in children up to 3 months of age may be much higher. Assuming this is 80%, the no-toxic effect level of nitrate derived from nitrite that should be used for extrapolation to young children is thus $100\%/80\% \times 10 \text{ mg/kg b.w.} = 12.5 \text{ mg sodium nitrate/kg b.w.}$ or 9.1 mg NO_3^- /kg b.w. In addition, young children under the age of 3 months are more vulnerable to methaemoglobin formation by nitrite than adults which means that the actual no-effect level applicable for infants may even be lower. In bottle-fed infants, the daily consumption of drinking water amounts to an average of 500 ml during the first weeks increasing to ca. 850 ml during the 2nd and the 3rd month (Nelson, 1983). At the present standard, drinking water may contain up to 50 mg NO_3^- /l. From these data, a realistic NO_3^- intake by infants weighing 3-5 kg can be calculated to be 8.3-8.5 mg/kg b.w./day.

This implies that there is hardly any margin of safety between intake and

no effect-level at longterm exposure for infants. However, some additional observations have to be made.

1. Various authors use 10% increased methaemoglobin as criterion for acute methaemoglobinemia in infants (Winton et al., 1971). At this methaemoglobin level cyanosis occurs. Shuval and Gruner (1972) found an increased methaemoglobin level (5%) of less than 10% in rats after longterm feeding of 100 mg/kg NaNO_2^- /kg b.w. Extrapolation of this dose level means that no (clinical) methaemoglobinemia would occur at 91 mg NO_3^- /kg b.w. in infants. The margin of safety (1) between actual intake and this so-called marginal-effect-level is a factor 11.
2. In practice, clinical cases of methaemoglobinemia in infants (cyanosis) were almost exclusively found in areas with a drinking water nitrate level of $\geq 44,3-88,6$ mg NO_3^- /l (WHO, 1985).

As a result of observations in infants, some authors suggest that the most important cause of methaemoglobinemia in infants is probably the endogenous synthesis of nitrate and nitrite due to gastrointestinal infections (Hegesh and Shiloa, 1982, and others). In some inflammatory states, an increased nitrate reduction to nitrite is indeed observed, sometimes -but not necessarily always- in combination with enhanced nitrate synthesis. However, this is only one of the possible causes of infant methaemoglobinemia. The results of Cornblatt and Hartmann (1948) confirm the findings from animals studies (oral uptake of nitrate results in increased methaemoglobin blood levels) also for healthy infants. Although the dose levels were high in this study, and no cyanosis occurred, methaemoglobin levels increased up to 7.5%, while the duration of exposure was only short and while most of the children were older than the critical age of 3 months.

As stated earlier, calculation of quantitative risks of longterm exposure has to be based on animals experiments. The possibly enhanced rate of

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- [1] For comparison: for calculation of an "acceptable daily intake" (ADI) from animal experiments, a safety factor is usually applied between intake and no-effect-level from longterm animal studies. This factor consists of a factor accounting for the uncertainties in extrapolation from animals to man (usually 10) and a factor accounting for individual sensitivity (also usually 10). In this case, infants are the most sensitive group; therefore, only the uncertainties in extrapolation from animals to man have to be taken into account.

endogenous nitrate synthesis, and especially the increased rate of reduction of nitrate to nitrite, in infants with gastrointestinal infections, give an added reason for limitation of exogenous uptake in this sensitive group.

For nitrite the average daily intake of man is estimated to be 2.3 mg NO_2^- /day or 38 $\mu\text{g/kg}$ b.w. thus the margin of safety for nitrite between intake and no-toxic effect level in animals is a factor 176, without inclusion of endogenously formed nitrite. Contrary to the intake of nitrate, there is no higher intake of nitrite by young children because the food commodities with relatively high nitrite concentrations are cured meats. As said before, infants are more vulnerable for methaemoglobin formation.

As indicated by the toxicity data summarized in this document, nitrate can be toxic by conversion to nitrite, but also by endogenous formation of carcinogenic N-nitroso compounds from nitrite and nitrosatable compounds (nitrosamines). To estimate the maximal cancer risk for lifetime ingestion of nitrate and nitrite and this endogenous nitrosation one has to deal with many uncertainties, as was indicated in this document. Next to the presence of nitrate or nitrite nitrosatable compounds e.g. amines have to be available concomittantly in adequate amounts. This was illustrated by studies with human volunteers fed a variety of diets containing nitrate which reveal traces of volatile nitrosamines (NDMA and NDEA) when vegetables rich in nitrate were consumed in combination with fish. In case of combined consumption of vegetables and meat little or no nitrosation is observed, as far as detection methods for nitrosamins are available (e.g. for volatile nitrosamines).

The formation rate of N-nitroso compounds under acid conditions varies for different amines and amides, and not all possibilities of nitrosation are studied. Because the endogenous formation of NDMA and NDEA in human beings has been demonstrated, the acceptable daily dose of these two nitrosamines for man (D_m) giving rise to a tumor incidence of $1:10^6$ is calculated, based on the most conservative one-hit model with linear extrapolation.

The D_m values are:

- nitrosodimethylamine (NDMA): $16-186 \times 10^{-6}$ mg (based on liver tumors),
- nitrosodiethylamine (NDEA): $11-14 \times 10^{-6}$ mg (based on liver tumors)
- and $7.5-13.5 \times 10^{-6}$ mg (based on liver and oesophagus tumors).

Based on an average nitrate and nitrite consumption in the Netherlands of respectively 110 mg NO_3^- /person/day and 2.3 mg NO_2^- /person/day the possible endogenous nitrosation of a variety of nitrosatable compounds can be calculated using the nitrosation figures of Ohshima and Bartsch (1981) who measured the amount of nitrosoproline in urine of man after ingestion of both nitrate and proline. Nitrosoproline is excreted in the urine for the major part unchanged. At intakes of 195 or 130 mg nitrate together with 500 mg proline ca. 3 en 2 μg nitrosoproline can be formed. At the intake of 65 mg nitrate together with 500 mg proline the amount of nitrosoproline was hardly detectable. It has to be noted that dimethylamine and diethylamine are nitrosated less rapidly than proline while morpholine and pyrrolidine are nitrosated more rapidly than proline. At the average daily intake of 110 mg NO_3^- /person it can be assumed that based on the nitrosation figures of Ohshima and Bartsch (1981), 2 μg nitrosamines/person/day can be formed from this amount of NO_3^- .

Assuming an average daily intake of 2.3 mg NO_2^- /person, it can be calculated, using the conversion rate of 5% of nitrate to nitrite, that this amount of NO_2^- had been formed from $100\%/5\% \times 2.3 \text{ mg} = 46 \text{ mg } \text{NO}_3^-$. According to Ohshima and Bartsch (1981) no detectable amounts of nitrosamines will be formed from this amount of NO_3^- . The estimated intake of preformed nitrosamines via food ranges from 0.6-1.4 μg /person/day, thus the oral daily exposure can amount to 2.6-3.4 μg /person/day.

The maximum calculated lifetime cancer risk based on the induction of liver tumors (in rats by diethylnitrosamine) for the intake of 1 μg of exogenous nitrosamines/person/day is 9×10^{-5} , but with the inclusion of endogenous nitrosation via ingested nitrate it may be $3 \times 9 \times 10^{-5} = 27 \times 10^{-5}$.

This risk evaluation is based on two conservative assumptions:

- 1) a relatively high endogenous nitrosation based on the nitrosation of proline from the study of Ohshima and Bartsch (1981).
- 2) the usage of a "one-hit" model with linear extrapolation for the calculation of the maximum lifetime cancer risk for man from animal data.

It is difficult or impossible to demonstrate a cause/effect relationship between exposure to low levels of nitrosamines and the incidence of certain cancers in the general population. Data from a number of epidemiologic studies are only suggestive. From epidemiologic studies in patients with stomach disorders or stomach diseases it appeared that within this group a

correlation between the induction of stomach cancer and the intake of relatively high amounts of nitrate could not be excluded. For people without stomach disorders or stomach diseases no distinct causal relationship between nitrate intake and the occurrence of stomach cancer was observed. In persons occupationally exposed to nitrate or nitrite no increased tumor incidences were found.

4.2 Ecosystems - aquatic organisms

The risk evaluation is restricted to nitrate, the main item of this document.

Because of the difference in toxicity between sodium nitrate and potassium nitrate (on the basis of the nitrate concentration) which has been found in short-term toxicity tests with freshwater organisms, the evaluation has been divided, if possible, into two sections, using either data on sodium nitrate or those on potassium nitrate.

At present there are no generally accepted methods for extrapolation of the results of laboratory "single-species" toxicity studies to natural ecosystems. Provisionally different theoretical procedures will be used to calculate "safe" concentrations for the aquatic environment. In this way information can be gathered on the usefulness of these methods of extrapolation. The results of these methods should only be considered indicatively.

In the method according to Slooff et al. (1986) either one short-term toxicity value (L(E)C50) or one long-term toxicity value (NOEC) is used to calculate a safe level. In both cases the value used is the lowest "reliable" value found in literature. In the method according to Kooijman (1985, 1987) either all available L(E)C50-values or all available NOEC-values are used. In the method according to Van Straalen (1986) - which has been derived from that according to Kooijman (1987) - all available NOEC-values are used. For more details, one is referred to the original publications.

Table 4.1 summarizes the results of these methods of extrapolation.

Final risk evaluation nitrate in surface water

The final evaluation is based on i) the risk evaluation on the basis of the single-species toxicity tests, see table 4.1, and ii) the appendix on eutrophication.

Freshwater

Based on data concerning eutrophication the evaluation with respect to freshwater has been split up in "oligotrophic water" (fens and other surface waters not fed by rivers) and "eutrophic waters" (rivers and surface waters fed by rivers).

Oligotrophic water

From the eutrophication point of view a concentration of $0.04 \text{ mg NO}_3^- \cdot \text{l}^{-1}$ is recommended. The toxicologically "safe" concentrations, calculated with the different methods of extrapolation, all are higher than this concentration. Therefore the concentration of 0.04 $\text{mg NO}_3^- \cdot \text{l}^{-1}$ is also considered to be safe for both individual species and ecosystems, at long-term exposure. On the basis of these data this concentration is proposed as maximum acceptable concentration in oligotrophic waters.

Eutrophic water

Because the above mentioned concentration of $0.04 \text{ mg NO}_3^- \cdot \text{l}^{-1}$ neither nowadays nor in the future situation can be considered as realistic for most surface waters in the Netherlands, another maximum acceptable concentration is proposed for these waters.

From the eutrophication point of view $1.8 \text{ mg NO}_3^- \cdot \text{l}^{-1}$ is recommended for rivers and surface waters fed by rivers. The toxicologically "safe" concentrations, calculated with the different methods of extrapolation, are ranging from 0.09 to $0.33 \text{ mg NO}_3^- \cdot \text{l}^{-1}$ on the basis of tests with potassium nitrate and from 0.12 to $2.56 \text{ mg NO}_3^- \cdot \text{l}^{-1}$ on the basis of tests with sodium nitrate. Most of these calculated concentrations are not considered to be realistic as maximum acceptable concentration in eutrophic waters. On the basis of all available data, taking into account the uncertainties in the methods of extrapolation used, a concentration of 2 $\text{mg NO}_3^- \cdot \text{l}^{-1}$ is provisionally proposed as maximum acceptable concentration in eutrophic water.

Seawater

From the eutrophication point of view $0.1 \text{ mg N} \cdot \text{l}^{-1}$ is recommended with regard to the combined amount of the nitrogen nutrients NO_3^- and NH_4^+ . If this amount of nitrogen would be completely in the form of nitrate, the value of $0.1 \text{ mg N} \cdot \text{l}^{-1}$ corresponds with about $0.4 \text{ mg NO}_3^- \cdot \text{l}^{-1}$. The toxicologically "safe" concentrations, calculated with the method according to Slooff et al. (1986) using short-term toxicity data, are 2.64 and $0.53 \text{ mg NO}_3^- \cdot \text{l}^{-1}$. Long-term data are lacking.

On the basis of these data 0.4 $\text{mg NO}_3^- \cdot \text{l}^{-1}$ is provisionally proposed as maximum acceptable concentration in seawater.

Table 4.1 "Safe" nitrate concentrations (in mg NO₃.l⁻¹) in the aquatic environment according to the methods of extrapolation proposed by Kooijman (1985,1987), Slooff et al. (1986) and Van Straalen (1986)

		freshwater	seawater
<u>SLOOFF ET AL.</u>			
NaNO₃			
I.	<u>L(E)C50 (short-term)</u>	<u>2.614</u>	<u>2.700</u>
I.	--> calculated NOEC _{ss} UF = 25.6 --->	65.53 "safe" 2.56	67.58 "safe" 2.64
I.	--> calculated NOEC _{eco} UF = 85.7 --->	44.46 "safe" 0.52	45.65 "safe" 0.53
II.	<u>NOEC (long-term)</u>	<u>5</u>	-
II.	--> calculated NOEC _{eco} UF = 33.5 --->	5.9 "safe" 0.18	
KN03			
I.	<u>L(E)C50 (short-term)</u>	<u>299</u>	-
I.	--> calculated NOEC _{ss} UF = 25.6 --->	8.35 "safe" 0.33	
I.	--> calculated NOEC _{eco} UF = 85.7 --->	7.68 "safe" 0.09	
II.	<u>NOEC (long-term)</u>	-	-
<u>KOOIJMAN</u>			
NaNO₃			
I.	<u>L(E)C50-values</u>	+	+
I.	--> "safe" L(E)C50-value @	121	147 (n = 1000 *)
II.	<u>NOEC-values</u>	+	-
II.	-->	"safe" 0.12 (n = 1000 *) 0.36 (n = 100 *) 1.05 (n = 10 *)	

----->

table 4.1 (continue)	freshwater	seawater

KN03

I. <u>L(E)C50-values</u>	+	-
--> "safe" L(E)C50-value @	0.30 (n = 1000 *)	
II. <u>NOEC-values</u>	-	-

VAN STRAALLEN

NaNO3

II. <u>NOEC-values</u>	+	-
II. -->	"safe"	1.78

KN03

II. <u>NOEC-values</u>	-	-

UF) "Uncertainty Factor"; ss) "single-species"; eco) "ecosystem".
 +) Data available; -) no data available.
 @) "Safe" L(E)C50-value: calculated value for most sensitive species.
 *) Extrapolated to the number of species mentioned.
 L(E)C50: Lethal-(Effect-) Concentration for 50% of the exposed organisms.
 NOEC: No-Observed-Effect-Concentration.

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Abbreviations and synonyms for most relevant nitroso compounds*

NAB	<u>N</u> -nitrosoanabasine
NAT	<u>N</u> -nitrosoanabatine
NDBA - DBN	<u>N</u> -nitrosodibutylamine - dibutylnitrosamine
NDEA - DEN	<u>N</u> -nitrosodiethylamine - diethylnitrosamine
NDELA	<u>N</u> -nitrosodiethanolamine
NDMA - DMN	<u>N</u> -nitrosodimethylamine - dimethylnitrosamine
NDPA - DPN	<u>N</u> -nitrosodipropylamine - dipropylnitrosamine
NMBA	<u>N</u> -nitrosomethylbenzylamine
NMOR	<u>N</u> -nitrosomorpholine
NNA	4-(<u>N</u> -nitrosomethylamino)-4-(3-pyridyl)butanal
NNK	4-(<u>N</u> -nitrosomethylamino)-1-(3-pyridyl)-1-butanone
NNN	<u>N</u> -nitrosornicotine
NPIP - NPip	<u>N</u> -nitrosopiperidine
NPYR - NPyr	<u>N</u> -nitrosopyrrolidine
TSNA	Tobacco-specific nitrosamines

* According to recommendations made at the 5th meeting on Analysis and Formation of N-Nitroso Compounds (Durham, N.H., U.S.A., 1977), as cited in IARC Publications no.45, 1983.

Table I: Conversion scheme for various methods of expressing nitrate and nitrite contents *

Nitrogenous substance	chemical formula	atomic or molecular weight	multiplication factors for the conversion from nitrogenous substance in column 1 to				
			NO ₃ -N	NO ₃	NH ₄ NO ₃	NaNO ₃	KNO ₃ **
nitrate-nitrogen	NO ₃ -N	14	1	4.43	5.70	6.07	7.21
nitrate	NO ₃	62	0.23	1	1.29	1.37	1.63
ammonium nitrate	NH ₄ NO ₃	80	0.18	0.78	1	1.06	1.26
sodium nitrate	NaNO ₃	85	0.16	0.73	0.94	1	1.19
potassium nitrate	KNO ₃	101	0.14	0.61	0.79	0.84	1
			multiplication factors for the conversion from nitrogenous substance in column 1 to				
			NO ₂ -N	NO ₂	NaNO ₂	KNO ₂	
nitrite-nitrogen	NO ₂ -N	14	1	3.29	4.93	6.07	
nitrite	NO ₂	46	0.30	1	1.50	1.85	
sodium nitrite	NaNO ₂	69	0.20	0.67	1	1.23	
potassium nitrite	KNO ₂	85	0.16	0.54	0.81	1	

* with additions, from Corré and Breimer (1979)

** Methods of expressing nitrate and nitrite contents

Nitrate may be used as various different compounds, in experiments as well as in its use as a food additive. Sodium nitrate, potassium nitrate and ammonium nitrate are the options. Nitrite may be applied as sodium or potassium nitrite. The quantities of nitrate compounds may be expressed as NO₃-N, NO₃ or as NH₄-, Na- or KNO₃. Nitrite compounds may be expressed as NO₂-N, NO₂ or as Na- or KNO₂. This may give rise to confusion due to the existing differences in molecular weight. Therefore, conversion factors from one to another compound are given.

Table II: Survey of short-term nitrate and nitrite experiments in animals

Species	Route	Dosage *	Results	Reference
rat	orally in diet	1, 2, 3, 4 or 6% KNO ₃ , 5% NaNO ₃ 4 weeks	at 2% KNO ₃ in females slightly increased methHb- levels, in males increased relative kidney weight	Til et al. (1985a,b)
rat F344	orally in diet	1.25, 2.5, 5, 10 and 20% NaNO ₃ 6 weeks	slightly or modera- tely reduced weight gain at autopsy abnormal spleen and blood colour	Maekawa et al. (1982)
rat	orally in drinking water	4000 mg NaNO ₃ /l, 14 months	-methHb levels same as controls, -moderate effect on plasma-vitamin E levels and incidence of pulmonary lesions	Chow et al. (1980)
rabbit	orally in gelatin capsules	200, 400 and 600 mg KNO ₃ /kg b.w., 4 weeks	within 2 weeks in- toxication symptoms as weight loss, tachycardia, polyuria and weakness	Nighat et al. (1981)
calf	orally in artificial milk	400, 2000, 5000 and 10000 mg NO ₃ /kg, 8 weeks	no abnormalities	Berende et al. (1977)
mouse	orally by stomach tube	110 mg NaNO ₂ per kg b.w.; 7 days	decreased heart function at forced running distance, ECG- and heart muscle abnormalities	Kinoshita et al. (1985)
mouse	orally in drinking water	100, 1000, 1500 and 2000 mg NaNO ₂ per liter; 3 months	decreased motoric activity; methaemo- globin levels up to 15%	Behrooz et al. (1971)
mouse	orally in drinking water	100, 1000, 1500 and 2000 mg NaNO ₂ per liter; 2 weeks	decreased motor activity	Grüner et al. (1971)

Table II Survey of short-term nitrate and nitrite experiments in animals (cont.)

Species	Route	Dosage *	results	Reference
rat	orally by stomach tube	40 and 80 mg NaNO ₂ /kg b.w. in one dose; 160 and 320 mg/kg b.w. in 8 doses of 20 and 40 mg/kg b.w.	in one dose: higher toxicity than divided over more doses; at once: considerably higher methHb levels	De Vries (1983)
rat	orally by stomach tube	-2x100 mg NaNO ₂ /kg b.w. at 2 hours interval -2x100 mg NaNO ₂ /kg b.w. at 4 hours interval	increased mortality no relevant disorders	Druckrey (1963)
rat	orally in drinking water	0.06, 0.125, 0.25, 0.5 and 1% NaNO ₂ ; 6 weeks	-slight and at 1% moderate growth depression -at 0.5 and 1.0% abnormal colour of blood and spleen by methaemoglobin -maximum tolerated dose 0.25% in drinking water	Maekawa et al. (1982)
rat	orally in drinking water	100, 300 and 2000 mg NaNO ₂ per liter; 2 months	EEG abnormalities; reversible only after 4 months at lowest dosage	Shuval et al. (1972)
male rat (1 month old)	orally in drinking water	200 mg NaNO ₂ per liter; 16 weeks	-methHb-levels 0.5-3.1% -higher incidence of pulmonary lesions	Chow et al. (1980)
rat	orally in diet	0.0093% NaNO ₂ ; 168 days	no effects on growth rate and organ weights	Tarr et al. (1942)
rat	orally in drinking water	170 and 340 mg/kg b.w.; 200 days	methaemoglobinaemia; increased spleen and heart weights; changed liver and kidneys weights	Musil (1966)

Table II Survey of short-term nitrate and nitrite experiments in animals (cont.)

Species	Route	Dosage *	results	Reference
male rat (2 months old)	orally in drinking water	2000 mg NaNO ₂ per liter; 14 months	-methHb-levels 1-35% -significant lower body and liver weights -lower plasma vitamin E levels -higher incidence of pulmonary lesions	Chow et al. (1980)
cat	orally in diet	0.039% NaNO ₂ ; 105 days	no effects on growth rate and organ weights	Tarr et al. (1942)
pig dog ruminants	intravenously	6 mg nitrite-N per kg b.w.	permanent methaemoglobinaemia	Jensen et al. (1941); Emerick et al. (1965)
chicken 1 day old	orally in diet	-0.4% KNO ₂ ; 2 weeks	-growth inhibition -enlargement of thyroid gland	Sell et al. (1963)
	orally in diet	-18-60 mg NaNO ₂ per kg b.w.**; 2 weeks	-decreased vitamin A deposits in liver	Brüggemann et al. (1964)

* abbreviations and symbols used

NaNO₃ = sodium nitrate

NaNO₂ = sodium nitrite

KNO₂ = potassium nitrite

**kg b.w. = kg body weight

Table III: Survey of chronic toxicity and carcinogenicity nitrate and nitrite experiments in animals (see also Birdsall, 1981)

Species	Route *	Dosage	Results	Reference
rat	orally in feed (NaNO ₃)	0.1, 1, 5 and 10%; 2 years	from 5% (slight) growth inhibition	WHO-JECFA (1962; 1974)
mouse	orally in drinking water (NaNO ₃)	12.3 g/l; 20-25 weeks, animals killed 10-13 weeks later	no increased lung tumor incidence	Greenblatt et al. (1973)
mouse	orally in diet (NaNO ₃)	25,000 and 50,000 mg/kg; life-time study	no increased tumor incidence	Sugiyama et al. (1979)
rat	orally in drinking water (NaNO ₃)	100 mg; 84 weeks, animals killed 20 weeks later	non-significant increase in pituitary adenomas in female rats	Lijinsky et al. (1973a)
rat	orally in feed (NaNO ₃)	2.5 and 5.0%; 2 years; animals killed 19 weeks later	-at highest dosage in females > 10% growth depression -hepato- and splenomegaly -in treatment groups no increase in tumour incidence	Maekawa et al. (1982)
rat	orally in drinking water (NaNO ₂)	100 mg/kg b.w.** long-term	decreased life span	Druckrey et al. (1963)
rat	orally in drinking water (NaNO ₂)	100, 1000, 2000 and 3000 mg/l; 2 years	at highest dose focal degeneration of heart muscle, fibrosis, thin and dilated coronary arteries; lung emphysema at 3 highest doses	Shuval et al. (1972)
mouse	orally in drinking water (NaNO ₂)	1-2 g/l, 20 weeks; animals killed after 10 weeks	no increased lung tumour incidence	Greenblatt et al. (1973)
mouse	orally in drinking water (NaNO ₂)	1000, 2500 and 5000 mg/l, 18 months	no tumours due to nitrite treatment	Inai et al. (1979)

Table III Survey of chronic toxicity and carcinogenicity nitrate and nitrite experiments in animals (see also Birdsall, 1981) (continued)

Species	Route*	Dosage	results	Reference
mouse	subcuta- neously	12 mg per mouse; 90 days, animals killed after 11 months	no increased lung tumours	Nakahara et al. (1959)
mouse	orally by stomach tube orally in drinking water	10 mg/kg b.w.** daily in last week pregnancy 500 mg/l through- out pregnancy	no increased lung tumours	Börzsönyi et al. (1978)
rat	orally in drinking water (NaNO ₂)	2 g/l, almost life-time	no increased tumours	Lijinsky et al. (1973a,b); Taylor et al. (1975)
rat	orally in drinking water	3000 mg/l, 5 days/weeks, life-time	papillomas of the fore-stomach in 8 out of 45 rats	Mirvish et al. (1980)
rat	orally in drinking water (NaNO ₂)	0.125 and 0.25%; 2 years: animals killed 16 weeks later	-highest dose in fe- males > 10% growth depression -hepato- and spleno- megaly -in treated males dose-related decrea- se in tumour inci- dence in comparison to controls	Maekawa et al. (1982)
rat	orally in diet (NaNO ₂)	males: 100 mg/kg b.w. females: 120 mg/kg b.w.	in both sexes a de- crease in mononuclear cell leukaemia	Lijinsky et al., 1983
	orally in drinking water (NaNO ₂)	males: 50 mg/kg b.w. females: 80 mg/kg b.w. 2 years		
rat	orally in diet (NaNO ₂)	1000 mg/kg	increased incidence of tumors of the lymphoreticular system	Shank et al. (1976)

Table III Survey of chronic toxicity and carcinogenicity nitrate and nitrite experiments in animals (see also Birdsall, 1981) (continued)

Species	Route*	Dosage	results	Reference
rat	orally in diet (NaNO ₂)	250, 500, 1000 and 2000 mg/kg; 1000 or 2000 mg/l	at 2000 mg growth inhibition	Newberne et al. (1978, 1979)
	orally in drinking water (NaNO ₂)	-prenatal -from age 21 days onwards	also at 2000 mg growth inhibition and to a lesser degree significant increase in lymphoic tumors	
rat	same study as above; evaluated by Government Interagency Working Group		no increased lymphomas, no increased other tumors	FDA (1980)
rat	orally in cured meat (NaNO ₂)	200, 1000 and 4000 mg/kg	tendency towards increased number of animals with tumors at 4000 mg; no effect on reproduction	Olsen et al. (1984)
rat	orally in drinking water	NMBA* 10 mg/l	increased progression of oesophageal tumors	Schweinsberg et al. (1985)
	orally in diet (NaNO ₂)	1 g/kg		
rat	orally in drinking water	100 mg/kg b.w.**; 3 generations	number of tumours not increased	Druckrey et al. (1963)
rat	orally in diet	1600 mg/kg; study terminated after 16 months	number of tumours not increased	Matsukura et al. (1977)
rat	orally in meal	2000 mg/kg; 78 weeks; study terminated after 27 months	no increased tumour incidence	Lijinsky et al. (1980)
rat	orally in cured meat	200 and 5000 mg/kg; 29 months	no increased tumour incidence	Van Logten et al. (1972)

* abbreviations and symbols used:
 NMBA - nitrosomethylbenzylamine
 NaNO₃ - sodium nitrate
 NaNO₂ - sodium nitrite
 **per kg b.w. - per kg body weight

Table IV: Survey of fetotoxicity and teratogenicity experiments with nitrate and nitrite

Species	Route *	Dosage	results	Reference
guinea pig	orally in drinking water (KNO ₃)	300, 2500, 10000 and 30000 mg/l, 143-204 days	decreased fertility, highly impaired mating behaviour and decreased number of pregnant animals at 30000 mg	Sleight et al. (1968)
guinea pig	orally in drinking water (KNO ₂)	300, 1000, 2000, 3000, 4000, 5000 and 10000 mg/l, 100-240 days	100% fetal mortality at 5000 and 10,000 mg/l; growth inhibition at 10,000 mg/l	Sleight et al. (1968)
guinea pig	subcutaneously (NaNO ₂)	50 and 60 mg/kg b.w.**	at 50 mg/kg partus normal; at 60 mg/kg increased fetal mortality and abortions; fetal deaths may be prevented by injection of 10 mg methylene blue/kg b.w.**	Sinha et al. (1971)
rat	orally in drinking water (NaNO ₂)	2000 and 3000 mg/l	anaemia, elevated methHb levels in pregnant animals; growth inhibition and increased mortality in offspring	Shuval et al. (1972)
rat	orally in feed (NaNO ₂)	3 and 10 g/kg	no embryotoxic or teratogenic effects	Alexandrov et al. (1971)
rat (2 generation study)	orally in diet (NaNO ₂)	240-460 mg/kg	no effects on litter size, neonatal mortality, life span or growth rate	Shank et al. (1976)
rat (teratogenicity study)	orally in meat (NaNO ₂)	0.2, 1, 4 g/kg	no effects on fertility, pre-implantational losses and resorptions; no change of litter size, sex ratio and average pup weight; no increased malformations	Carstensen et al. (1972)

Table IV Survey of fetotoxicity and teratogenicity experiments with nitrate and nitrite (continued)

Species	Route *	Dosage	results	Reference
sheep	orally in hay	11% NO ₃ -N	at doses giving high methHb levels: no increased number of abortions	Davison et al. (1965)
	orally in drinking water	0.07, 0.14, 0.28% NO ₃ -N; 41-74 days, until parturition		
cow	orally on chopped hay	-4 cows on sodium nitrate leading to 20-30% methHb -9 cows on sodium nitrate leading to 40-50% methHb -4 cows on sodium nitrite leading to 40-50% methHb (between 100 and 150 mg NO ₃ -N/kg) 7 months	at doses giving 20-30% methHb levels 40-50% methHb levels: -no effect on reproductive capacity -no increased number of abortions	Winter et al. (1964)

* abbreviations symbols used:

NaNO₂ = sodium nitrite

KNO₂ = potassium nitrite

KNO₃ = potassium nitrate

** kg b.w. = kg body weight

Table V Survey of epidemiological studies relating nitrate level in drinking water, food or occupation to gastric cancer

route of nitrate exposure	population origin	number of persons in each area	parameters (s)	results and correlation with gastric cancer	authors(s)
drinking water	202 urban areas in 25 provinces Chile	total population	- nitrate level in drinking water - gastric cancer death rate	- nitrate level from 0-30 mg/l; in 2 provinces > 11.3 mg/l - no association nitrate level and gastric cancer	Zaldivar and Wetterstrand, 1978
drinking water	population Aalborg and Aarhus Denmark	total population	- nitrate level in drinking water - gastric cancer mortality	- Aalborg nitrate level 6.8 mg NO ₃ -N/l - Aarhus - lower level - no correlation	Jensen et al., 1982
drinking water	- mining town, former nitrate level of 90 mg NO ₃ -/l - 5 nearby mining towns - 4 non-mining towns, England	whole population statistics on death rate from gastric cancer	- death rate from gastric cancer - calculated nitrate uptake from assumed food and water consumption	- urinary nitrate levels mining town higher than in controls - positive association between nitrate level and gastric cancer for females	Hill et al., 1973
drinking water	same population as above	total population as above	- mortality for stomach cancer during 1958-1975	- data corrected for mining area and social class - gastric cancer mortality in women - not significantly - raised; no correlation	Davies, 1980
drinking water	253 urban areas in England	total population	- mortality data for 1969-1973 in relation to water intake	- no association, even when analysis was restricted to urban areas with nitrate levels above the EC-guideline (50 mg NO ₃ -/l)	Beresford, 1985
drinking water	753 communities in N. France	total population	- digestive and urinary cancer incidence (1968-1975) - nitrate concentration measured during 1974-1976	- mean nitrate concentrations below 95 mg NO ₃ -/l, 93% under 43 mg NO ₃ -/l - no association between nitrate concentration and digestive and urinary cancer mortality rates	Vincent et al., 1983
drinking water	population of 230 localities in county of Szabolcs-Szatmar Hungary	total population	- gastric cancer incidence - nitrate level in well water	- no correlation	Juhasz et al., 1980
drinking water	human volunteers	25 and 10	- salivary nitrite level	- drinking water with 50 mg NO ₃ -/l: no significant changes in salivary nitrite levels - drinking water with 200 mg NO ₃ -/l: increase in salivary nitrite levels consistently found	Hart and Walters, 1983

Table V Survey of epidemiological studies relating nitrate level in drinking water, food or occupation to gastric cancer (continued)

route of nitrate exposure	population origin	number of persons in each area	parameters (s)	results and correlation with gastric cancer	authors(s)
food and drinking water	school children 11-13 years in Chile	relevant population: - 2 areas of high - 2 areas of low stomach cancer mortality	- urinary nitrate levels - salivary nitrite levels - levels of nitrate and nitrite in selected vegetables	- levels of urinary nitrate higher in children from low-risk area - levels of salivary nitrite similar in both areas no relation nitrate intake and stomach cancer incidence	Armijo et al., 1981b
food	residents visiting hospital patients from 2 high- and 2 low-risk areas for gastric cancer in England	between 200 and 250 for each area	- level of nitrate and nitrite in saliva (for estimation of exposure)	- low-risk population had significantly increased salivary nitrate and nitrite levels - inverse trend of the one for stomach cancer	Forman et al., 1985
environmental exposure by natural fertilizers	25 provinces in Chile	population of provinces	- death rates of stomach cancer mortality during 1957-1971 - data concerning nitrate usage for period 1945-1972	- high correlation between death rates and cumulative per capita exposure to nitrogen fertilizers	Armijo et al., 1975
environmental pollution by natural fertilizers	25 provinces in Chile	population of provinces mentioned	- nitrates per unit area (kg/ha) - stomach cancer death rate	- significant association between nitrate load and gastric cancer	Zaldivar, 1977
occupational	fertilizer manufacturers	- data base from 2 cohorts on men aged 35-64 between 1961-1971 as manufacturers of fertilizers or chemicals for pest control	- cancer mortality - frequency of exposure to nitrate containing dust	- weak evidence of association between cancer mortality and frequency of exposure to nitrate containing dust	Fraser et al., 1982

Table VI Survey of epidemiological studies relating nitrate level in persons with gastric complaints or disorders

route of nitrate exposure	population origin	number of persons in each area	parameters (s)	results and correlation with gastric cancer	authors(s)
food (diet)	Toronto, Winnipeg and St. Johns (Canada)	- 246 histologically verified gastric cancer cases - 246 matched controls	- daily nutrient consumption calculated from diet questionnaire data	- decreased trend in risk with calculated nitrate consumption - average daily nitrate consumption associated with increasing trend in risk of gastric cancer - citrus fruit somewhat protective, vitamin C less so, and vitamin E not at all	Risch et al., 1985
occupation in agriculture (via fertilizers)	patients of 7 clinics in agricultural provinces with very high incidence for stomach cancer in Chile	- 360 cases of confirmed adenocarcinoma - 360 matched controls	- stomach cancer incidence - agricultural occupation	- longer-term residence in high-risk areas in early life by cases in comparison to controls - association between stomach cancer and prior occupation in agriculture	Armijo et al., 1981a
diet	population of 40,824 individuals from 6 high- and 4 low-risk areas for stomach cancer (China)	from resulting 8644 cases with chronic stomach complaints 3789 underwent biopsy of gastric mucosa (1995 males and 1794 females)	- gastric biopsy - nitrate and nitrite in saliva and gastric juice - pH, free acid content, total number of bacteria and presence of fungi in stomach contents	- incidence of atrophic gastritis increased in high-risk areas and significantly correlated with mortality for stomach cancer - in patients from high-risk area free acid concentration of gastric juice was decreased, while pH, number of nitrate reducing bacteria, fungi and the nitrite concentration were significantly elevated	Ru-fu et al., 1984
food and drinking water	patients from hospitals in Narino (Colombia) from 2 low-risk and 2 high-risk areas for stomach cancer	- 276 patients with stomach cancer - 276 matched controls - 173 sources of water supplies	- nitrate concentration in well water - salivary nitrate + urinary nitrite	- low nitrate level in water - positive correlation of gastric cancer risk and prevalence of chronic atrophic gastritis, nitrate content of well waters and nitrate excretion by the population - urinary excretion reflected nitrate ingestion	Cuello et al., 1976
	population from high mountainous areas of Andes with very high risk of gastric cancer (Colombia)	- 55 patients with previously undefined gastric pathology - 18 patients with diagnosed gastric pathology related to a precancerous state	- stomach pH < 5 in comparison to pH > 5 - gastric pH, nitrite and nitrate	- at pH > 5 a 20-100 fold higher nitrite concentration in gastric fluid - patients with diagnosed gastric pathology related to a precancerous state shown to have high levels of a putative carcinogen precursor	Tannenbaum et al., 1979

Table VI Survey of epidemiological studies relating nitrate level in persons with gastric complaints or disorders (continued)

route of nitrate exposure	population origin	number of persons in each area	parameters (s)	results and correlation with gastric cancer	authors(s)
food, drinking water and life-style	patients with stomach complaints (England)	- 217 patients with common upper gastro-intestinal complaints - 50 healthy controls	- in fasting gastric juice: - nitrite levels - concentration of N-nitroso compounds - pH - concentration of nitrate reducing bacteria	- relation between pH, nitrite, nitrate reducing bacteria and N-nitrosamines in fasting gastric juice - possible relation to gastric cancer	Reed et al., 1981a
	patients with stomach complaints (England)	- 140 patients taking cimetidine - 267 persons (including 50 healthy volunteers) not taking cimetidine	- in fasting gastric juice: - nitrite levels - concentration of N-nitroso compounds - pH - concentration of nitrate reducing bacteria	- in cimetidine treated patients significantly higher concentrations of N-nitroso compounds and pH levels; gastric nitrite level often raised - possible relation to stomach cancer	Reed et al., 1981b
	patients with pernicious anemia (PA) and controls (England)	- 13 fasting patients with PA - 13 age-matched controls	- in fasting gastric juice: - nitrite concentration - number of bacteria	- mean nitrite concentration nearly fifty-fold increased in PA patients - number of bacteria also greatly increased - possible relation to stomach cancer	Ruddell et al., 1978
	- 108 patients undergoing gastroscopy (Fed. Republ. Germany) - 20 duodenal ulcer - 11 benign gastric ulcer - 32 inflammatory diseases gastric mucosa - 19 atrophic gastritis - 26 controls	- nitrite and nitrosamine levels in gastric juice	- nitrite and nitrosamine levels significantly elevated in the patients with atrophic gastritis - relationship between constant exposure of damaged gastric mucosa to N-nitroso compounds and high incidence of gastric cancer in atrophic gastritis	Schlag et al., 1982	
diet	56 individuals before and after gastric operation (Greece)	56 persons	- in gastric juice: - pH - total number of nitrate reducing bacteria - nitrite level	- mean pH of post-operative samples significantly higher than pre-operative samples - positive correlation with pH and concentration of nitrate reducing bacteria and nitrite	Kyrtopoulos et al., 1985a

Table VII Environmental exposure of human beings to preformed nitrosamines

source of exposure	nitrosamine identified*	concentration range	estimated intake/person ug/day/person
food	NDMA	variable, depending	0.5-1.2
	NPYR	on food item	0.1-0.2
cosmetics	NDELA	1-48000 ug/kg	0.4
drugs and pesticides	NDMA	1-330 ug/kg	?
household commodities:			
surfactants	volatile	46-150 ug/kg	?
rubber products		1-280 ug/kg	?
air pollution			
indoor	NDMA	10-130 ug/m3 (cig. smoke)	?
outdoor		< 1 mg/m3	?
cigarette smoke (main stream)	NNN	0.2 -3.7	
	NNK	0.1 -0.44 ng/cig.	± 17
	NAB	0.15	
	NAT+others	0.14-4.6	
snuff	NNN	0.8 -33	
	NNK	0.2 - 4.6 mg/kg	± 200
	NAB	0.01- 1.9	
	NAT	0.2 -40	
	NDELA	0.3 - 3.3 mg/kg	

* NNK - 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone

NNN - nitosonornicotine

NDMA - nitrosodimethylamine

NAB - nitrosoanabasine

NPYR - nitrosopyrrolidine

NAT - nitrosoanabatine

NDELA - nitrosodiethanolamine

Table VIII a Results of mutagenicity studies with 14 nitrosamines

	bacteria		fungi		Drosophila				plants	mammalian systems in vitro					
	gene-mutations	DNA damage	gene-mutations	DNA damage	s x rl	chromosome loss	translocations	somatic mutations		gene-mutations	chromosomal aberrations	SCE	DNA		
													synthesis inhibition	damage	repair
nitroso-															
dimethylamine	+	+	+		+	+	+	+	+	+	+	+	+	+	+
diethylamine	+	+	+	+	+	±	+	+	+	+	+		+	+	+
dipropylamine	+		+					+		+				+	+
dibutylamine	+	+	±						+	+			+	+	+
piperidine	+	±	+	±	+	±		+		+			+	+	+
morpholine	+	±	+	-	+		+	+	-	+	±	+		+	+
pyrrolidine	+	+			+					+				+	+
thiazolidine	±	+												+	
diethanolamine	±	-			±			+							
NNK	+													+	
nicotine	+													+	
anabasine															
anabatin															
NNA															

± = positive and negative results

TP = transplacental

NNK = 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone

NNA = 4-(N-nitrosomethylamino)-4-(3-pyridyl)butanal

Table IX Animal species, in which 14 nitrosamines were studied for tumour induction

	DMN	DEN	DpN	PBN	NPip	NM	NPyr	nitrosothiazolidine	NDEIA	NNK	NNN	NAB	NAT	NAA
Unio	+	+												
Rainbow trout	+	+												
Zebra fish	+	+												
Medaka		+												
Guppy	+	+												
Poeciliopsis lucida		+												
Rivulus marmoratus		+												
Palmed newt	+	+												
Northern clawed frog	+	+												
Grass frog	+	+												
Python reticulatus		+												
Duck	+	+												
Chicken		+												
Grass parakeet		+												
Algerian hedgehog		+												
European hamster	+	+		+	+	+	+		+	+	+			
Syrian golden hamster	+	+		+	+	+								
Chinese hamster	+	+												
Dzungarian hamster		+												
Gerbil		+												
White tailed rat		+												
Praomys	+	+												
Mouse	+	+												
Rat	+	+												
Guinea-pig	+	+	+	+	+	+	+			+	+	+	*	+
Blue fox	+	+												
Mink	+	+												
Dog		+												
Cat		+												
Rabbit		+												
Pig	+	+												
Thick-tailed bushbaby		+												
Capucin monkey		+												
Grass monkey		+												
Rhesus monkey		+												
Cynomolous monkey		+												

* study was considered to be inadequate

Based on: IARC (1978, 1985)
Schmahl and Habes (1980)
Bogovski and Bogovski (1981)
Koppang et al. (1981)

Schultz and Schultz (1982)
Schmahl and Scherf (1983)
Kornig and Chasar (1984)

Table X Main target organs in 5 animal species

nitrosamine	mouse	rat	guinea-pig	Syrian hamster	Euroean hamster
DMN	liver, lung, kidney	liver, kidney, oesophagus, forestomach	liver	liver, nasal cavity	liver
DN	liver, oesophagus, forestomach, lung	liver, upper gastrointestinal tract (forestomach, oesophagus, tongue) kidney	liver	upper respiratory tract, liver	liver, lung
DPN	-	liver, upper gastrointestinal tract (oesophagus, tongue) kidney	-	upper respiratory tract	-
DBN	bladder, liver, oesophagus, forestomach, lung	bladder, liver, forestomach, oesophagus, lung, nasal cavity	liver, bladder	lung, bladder	respiratory tract, forestomach, bladder
NPip	liver, lung, forestomach, oesophagus	liver, oesophagus, respiratory systems	-	nasal cavity, respiratory tract	nasal cavity, lung forestomach
NM	liver, lung	liver, kidneys, blood vessels	-	respiratory system (nasal cavity, trachea, larynx)	respiratory system (nasal cavity, trachea)
NPyr	liver, lung	liver	-	liver, respiratory tract	-
nitroso-thiazolidine	-	-	-	-	-
NDEA	-	liver, kidney, nasal cavity	-	nasal cavity, liver, upper respiratory tract	-
NNK	lung	nasal cavity, lung, liver	-	trachea, lung, nasal cavity	-
NNN	lung	upper digestive tract (oesophagus), nasal cavity	+	nasal cavity, trachea	-
NAB	-	oesophagus	-	-	-
NAT	-	-	-	-	-
NNA	lung (inadequate study)	-	-	-	-

- = no (adequate) studies available Based on: IARC (1978); Preussmann and Stewart (1984); Hoffmann et al. (1985)

Table XI Toxicity to freshwater organisms - nitrate (short-term)

organisms	temp.	test subst.	medium	S/R/CF*	A**	time (hrs)	criteria	results (NO ₃ ⁻ mg.l ⁻¹)	references
<u>Mollusca/Gastropoda</u>									
<i>Lymnaea</i> sp. (eggs)	-	NaNO ₃	lake water, filtered	-	-	24, 72, 96	EC50 (hatching)	4654, 4343, 2373	Dowden and
	-	KNO ₃	lake water, filtered	-	-	24, 72	EC50 (hatching)	1184, 621	Bennet, 1965
<u>Arthropoda/Crustacea</u>									
<i>Daphnia magna</i>	-	NaNO ₃	lake water, filtered	-	-	24, 48, 72, 96	LC50	4365, 2614, 1551, 485	Dowden and
	-	NaNO ₃	standard reference water, free from organics	-	-	96	LC50	3070	Bennet, 1965
		KNO ₃	lake water, filtered	-	-	24, 48, 72, 96	LC50	299, 299, 138, 24	Dowden and
		KNO ₃	standard reference water, free from organics	-	-	100	LC50	549	Bennet, 1965
<u>Chordata/Pisces</u>									
<i>Carassius carassius</i>	-	NaNO ₃	standard reference water, free from organics	-	-	24	LC50	8870	Dowden and
									Bennet, 1965
<i>Lepomis macrochirus</i>	-	NaNO ₃	idem	-	-	24	LC50	9344	Dowden and
		KNO ₃		-	-	24	LC50	3355	Bennet, 1965
<i>Lepomis macrochirus</i>	20 °C	NaNO ₃	-	-	-	96	LC50	8760	Westin, 1974
		KNO ₃	-	-	-	96	LC50	1830	(primary source Trama, 1954)
<i>Poecilia reticularis</i> (fry, 7-11 mm)	25 °C	KNO ₃	tap water, aerated, DO 8.3 mg.l ⁻¹ , hardness (as CaCO ₃) 117-126 mg.l ⁻¹ , pH 7.4-7.7	S	A	24, 48, 72, 96	LC50 (average 2-4 groups)	1180, 970, 880, 850	Rubin and Elmaraghy, 1977
<i>Ictalurus punctatus</i> (fingerlings, 50-76 mm)	22 °C 26 °C 30 °C	NaNO ₃	aerated water, DO near saturation, hardness (as CaCO ₃) 102 mg.l ⁻¹ , pH 6.8-8.8	S	-	96	LC50 (threshold)	6000 6300 6200	Colt and Tschobanoglous, 1976
<i>Oncorhynchus tshawytscha</i> (fingerlings, 52-100 mm)	14-17 °C	NaNO ₃	reconstituted de-ionized fresh water, aerated, DO near saturation	R (after 96 hrs)	-	96, 168	LC50	5800, 4800	Westin, 1974
<i>Salmo gairdneri</i> (fingerlings, 52-115 mm)	13-15 °C	NaNO ₃	reconstituted de-ionized fresh water, aerated, DO near saturation	R (after 96 hrs)	-	96, 168	LC50	6000, 4700	Westin, 1974

* S = static; R = renewal; CF = continuous flow

** A = concentration of test compound analysed during assay

- = no data

Table XII Toxicity to freshwater organisms - nitrate (long-term)

organisms	temp.	test subst.	medium	S/R/CF*	A**	time (days)	criteria	results (NO ₃ ⁻ mg.l ⁻¹)	references
<u>Chordata/Pisces</u>									
<i>Ictalurus punctatus</i> (10-15 cm at start)	-	nitrate	increasing nitrate concentration, from 300 mg.l ⁻¹ at start to 400 mg.l ⁻¹	CF	A	0 → 60	NOEC (mortality, growth, feeding activity)	300 → 400	Knepp and Arkin, 1973
<i>Micropterus salmoides</i> (7.5 cm at start)	-	nitrate	increasing nitrate concentration, from 170 mg.l ⁻¹ at start to 400 mg.l ⁻¹	CF	A	0 → 164	NOEC (mortality, growth, feeding activity)	170 → 400	Knepp and Arkin, 1973
<i>Oncorhynchus tshawytscha</i> (eggs up to fry)	10 °C	NaNO ₃	DO 8-10 mg.l ⁻¹ , hardness (as CaCO ₃) 25 mg.l ⁻¹ , pH 6.2	CF	-	up to 30 days past yolk absorption	NOLC	> 20 (eggs), 10 (fry)	Kincheloe et al., 1979
<i>Salmo gairdneri</i> , steelhead trout (eggs up to fry)	10 °C	NaNO ₃	idem	CF	-	idem	NOLC	< 5 (eggs), ≥ 20 (fry)	Kincheloe et al., 1979
<i>Salmo gairdneri</i> , rainbow trout (eggs up to fry)	10 °C	NaNO ₃	idem	CF	-	idem	NOLC	5 (eggs), 5 (fry)	Kincheloe et al., 1979
<i>Oncorhynchus kisutch</i> (eggs up to fry)	10 °C	NaNO ₃	idem	CF	-	idem	NOLC	> 20 (eggs), ≥ 20 (fry)	Kincheloe et al., 1979
<i>Salmo clarki</i> (eggs up to fry)	13 °C	NaNO ₃	DO 6-9 mg.l ⁻¹ , hardness (as CaCO ₃) 39 mg.l ⁻¹ , pH 7.6	CF	-	idem	NOLC	10 (eggs), 20 (fry)	Kincheloe et al., 1979

* S = static; R = renewal; CF = continuous flow

** A = concentration of test compound analysed during assay

- = no data

Table XIII Toxicity to marine organisms - nitrate (short-term and long-term)

organisms	temp.	test subst.	medium	S/R/CF*	A**	time	criteria	results (NO ₃ ⁻ mg.l ⁻¹)	references
<u>Algae</u> Ten species of estuarine benthic diatoms	12 °C	KNO ₃	artificial seawater, salinity 15 or 34 ‰, pH 8.0	S	-	7 days	NOEC (growth) 30-65% growth inhibition	418 (all species) 1048 (5 species; other 5 species not affected)	Admiraal, 1977
<u>Annelida/Polychaeta</u> Ophryotrocha diadema	21 °C	HNO ₃	sterilized seawater, salinity 32 ‰	S	-	48 hours	LC50	32.5-98.4	Parker, 1984
<u>Mollusca/Lamellibranchiata</u> Mercenaria mercenaria (4.7-5.2 mm (juveniles); 28-32 mm (adults))	20 °C	NaNO ₃	seawater, salinity, 35 ‰, aerated, DO 7.0-8.2 mg.l ⁻¹ , pH 7.7-8.2	S	A	96 hours 20 hours	LC50 NOEC, EC (inhibition of clearing of algae from suspension)	> 19900 (j + a) 4960 (j + a) 9920 (j + a)	Epifanio and Sma, 1975
Crassostrea virginica (13-19 mm (juveniles); 46-62 mm (adults))	20 °C	NaNO ₃	idem	S	A	96 hours 20 hours	LC50 NOEC, EC (inhibition of clearing of algae from suspension)	16800 (j); 11500 (a) 4960 (j + a) 9920 (j + a)	Epifanio and Sma, 1975
<u>Chordata/Pisces</u> Gaidropsarus capensis (larvae, 105-155 days)	15 °C	NaNO ₃	seawater, salinity 34-38 ‰, DO 7.9-8.1 mg.l ⁻¹ , pH 7.8-7.9	S	-	24 hours 24 hours	LC50 EC50, EC10 (first-feeding incidence)	> 17700 4500, 1600	Brownell, 1980
Heteromyceteris capensis (larvae, 135-183 days)	15 °C	NaNO ₃	idem	S	-	24 hours 24 hours	LC50, LC10 EC50, EC10 (ffi)	22400, 15500 3100, 1900	Brownell, 1980
Diplodus sargus (larvae, 135-183 days)	15 °C	NaNO ₃	idem	S	-	24 hours 24 hours	LC50, LC10 EC50, EC10 (ffi)	15800, 13700 3500, 1200	Brownell, 1980
Lithognathus mormyrus (larvae, 134-182 days)	15 °C	NaNO ₃	idem	S	-	24 hours 24 hours	LC50, LC10 EC50, EC10 (ffi)	15300, 13400 2700, 1200	Brownell, 1980
Oncorhynchus tshawytscha (fingerlings, 52-100 mm)	13-14 °C	NaNO ₃	artificial seawater R (after 96 hrs) (15 ‰), aerated DO near saturation	-	-	96, 168 hours	LC50	4400, 4000	Westin, 1974
Salmo gairdneri (fingerlings, 52-115 mm)	13-14 °C	NaNO ₃	artificial seawater R (after 96 hrs) (15 ‰), aerated DO near saturation	-	-	96, 168 hours	LC50	4650, 4000	Westin, 1974

* S = static; R = renewal; CF = continuous flow

** A = concentration of test compound analysed during assay

- = no data

Figure I Fate of nitrate and nitrite in mammals (Vogtmann, 1985; adapted)

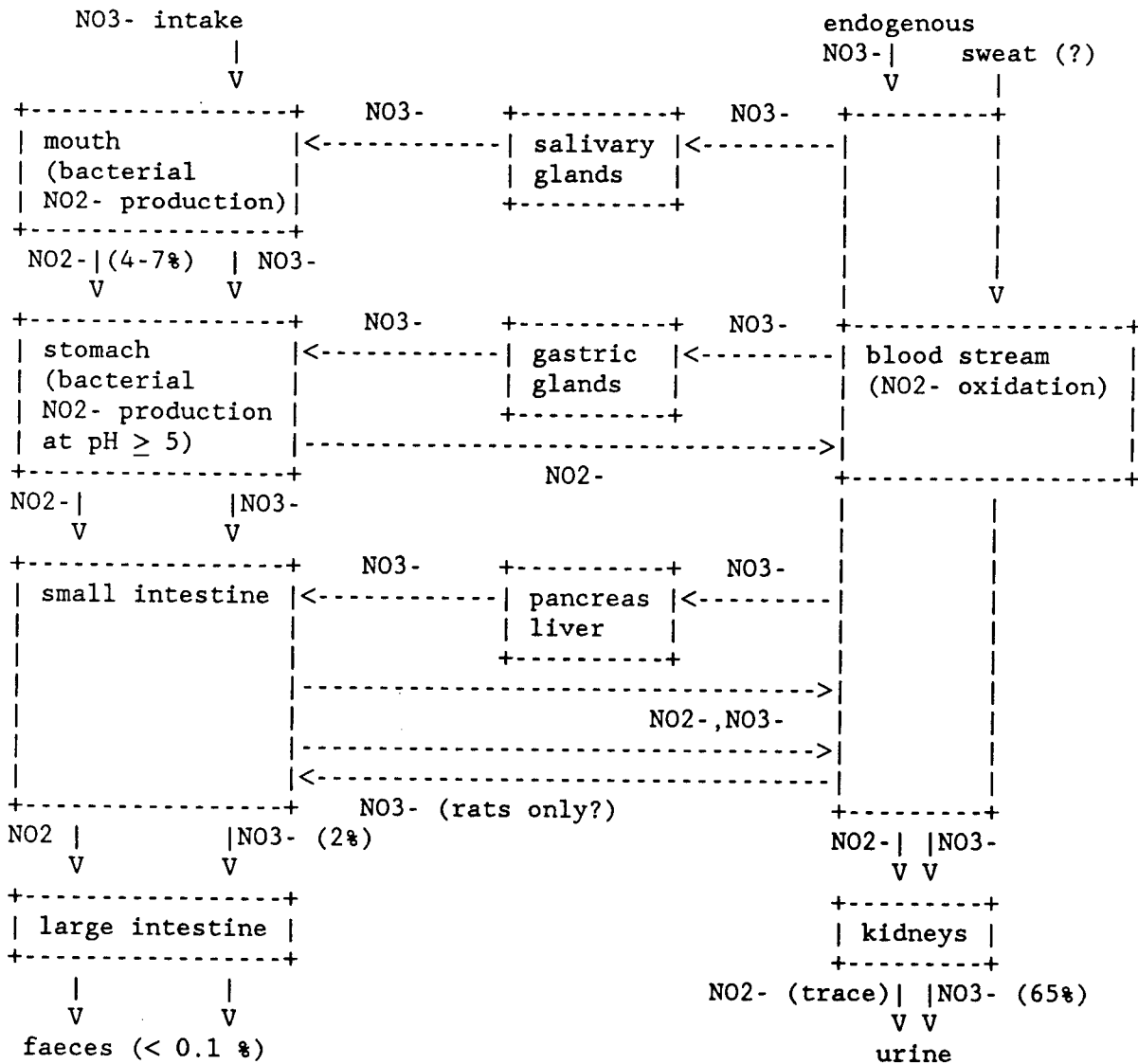


Figure II Proposed metabolism of nitrosamines

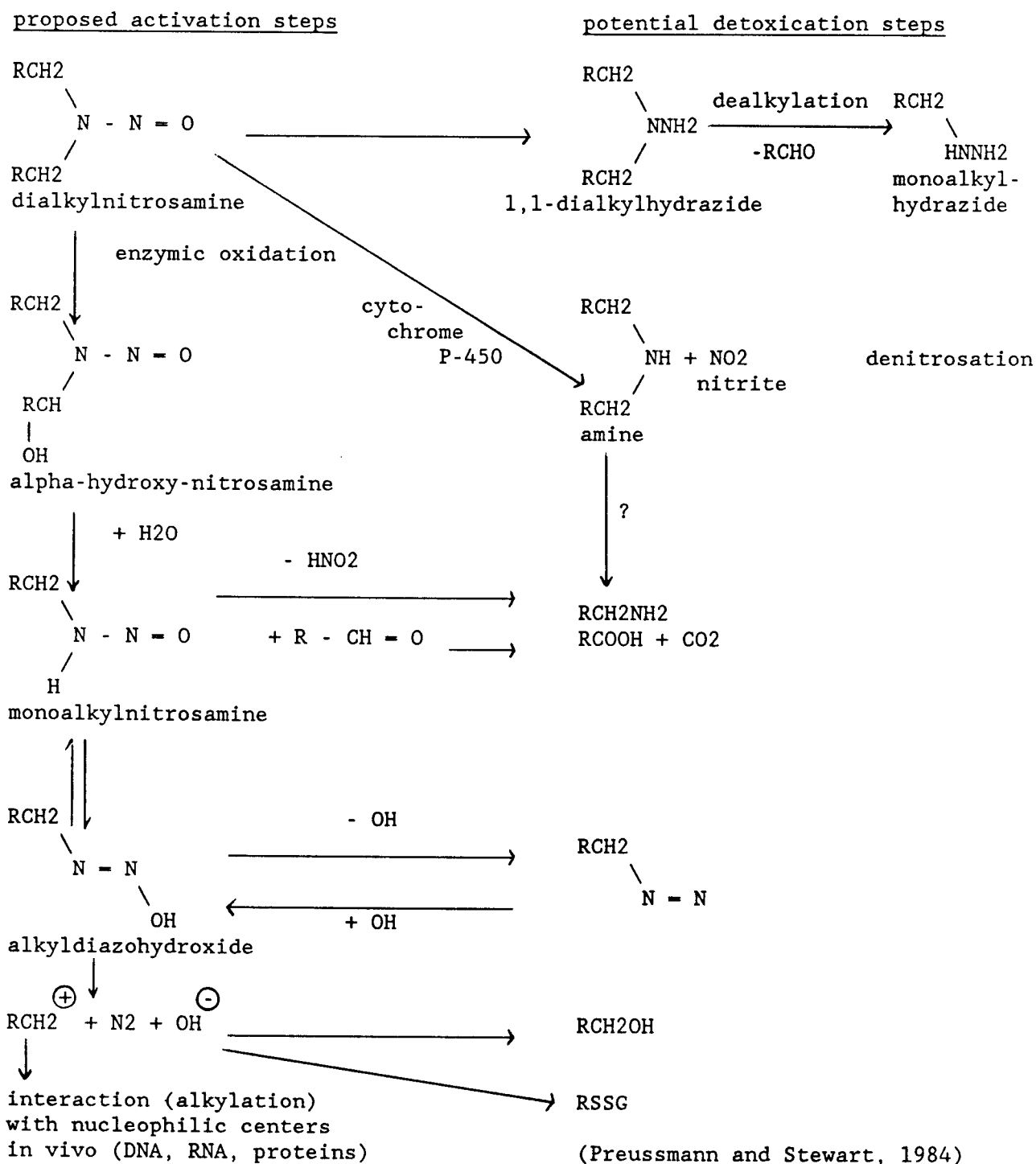


Figure III Alkylation of purine and pyrimidine bases

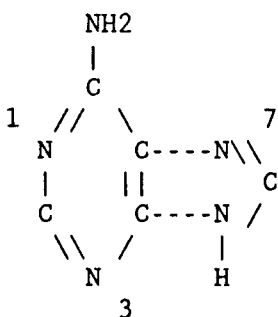
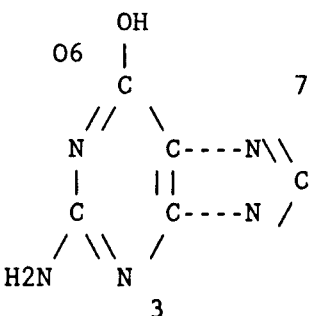
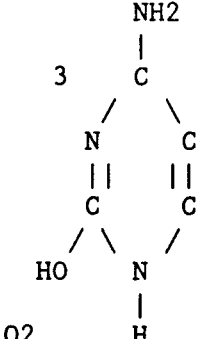
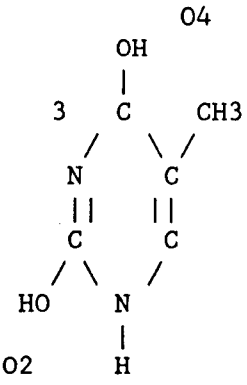
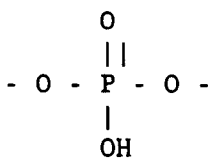
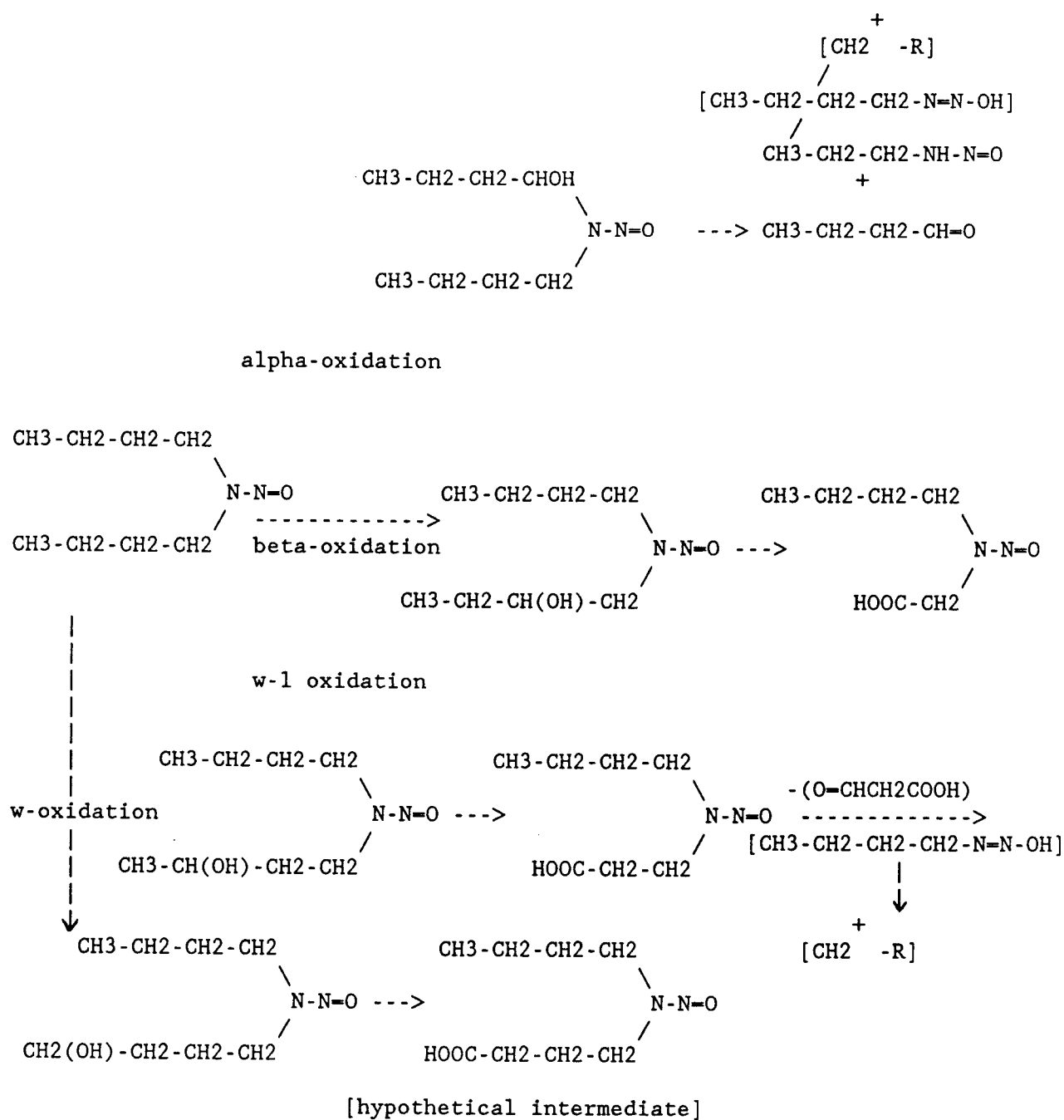
<u>base</u>		<u>positions alkylated</u>
adenine		1, 3, 7
guanine		3, 7, O6
cytosine		3, O2
thymine		3, O2, O4
phosphate		Pegg (1983)

Figure IV Pathways for the biotransformation of dibutyl nitrosamine



Eutrophication of surface waters

Strictly speaking the phenomenon eutrophication is characterized by an increase of the input and concentration of mineral plant nutrients. Increasing the nutritional state of a surface water results in an enhanced primary production, an increase of autotrophic biomass and, ultimately, in the excessive occurrence of only a single species of algae (plankton bloom). In more applied terms both cause and effects are addressed as eutrophication.

In counter-acting the effects of eutrophication it is of the utmost importance to know which of the nutrients is limiting algal growth in a given situation. In this respect, phosphorus and nitrogen are considered to be the most important elements. In organisms the average nitrogen:phosphorus ratio is 16:1, whereas in riverine systems in The Netherlands this ratio is between 18 and 36:1. From this it can be concluded that algal growth in rivers is limited by the availability of phosphorus. Since most of the surface water in The Netherlands are flushed with water from the river Rhine it is expected that the same type of limitation occurs. However, of some excavated peat lakes (Rijnland) flushed with water of the river Rhine it is established that nitrogen is consequently the limiting factor for algal growth (Schmidt van Dorp, 1978; Zevenboom, 1980). For coastal areas the N:P ratio is hard to assess. The inflow of riverine fresh water which is P-limited and the fact that the atmospheric deposition of nitrogen containing compounds is considerable in contrast to the deposition of phosphorus leads to the conclusion that coastal waters will probably be P-limited too.

The nitrogen cycle.

In assessing the fate of nitrogen in the environment some information is needed on the bio-geo-chemical cycling of nitrogen. The transformational processes underlying the nitrogen cycle are the same in both soil and aquatic systems (De Kruijf et al., 1984). The process of primary production incorporates inorganic nitrogen (nitrate and ammonium) in simple and complex organic constituents of plant tissue. The nitrogen present in vegetable proteins and nucleotids is providing food for

herbivores. Microbial degradation of vegetable and animal material will release nitrogen in the reduced form as ammonium. In the aerobic process of nitrification specialized bacteria stepwise oxydize ammonium to subsequently nitrite and nitrate. Under anaerobic conditions denitrifying bacteria convert nitrate to nitrogen- and nitrous oxyde gas (N_2O) by oxydizing organic substrate with the aid of the oxygen contained in nitrate. Nitrous oxyde is badly soluble in water and enters the atmosphere where it can harm the ozone layer and thus may add to the hot-house effect (Helder, 1983). Nitrogen gas, which is the end-point of denitrification, is biologically inactive, since it can only reenter the food chain by mediation of certain nitrogen fixing bacteria and cyano-bacteria. Normally nitrification and denitrification occur simultaneously or subsequently in every system. In a balanced environment these processes assure that excess amounts of nitrogen nutrients are eliminated. Throughout the year the domination of the individual inorganic nitrogen compounds will show differences which are mainly governed by temperature (biological activity) and the availability of oxygen. The decomposition of organics to ammonium takes place in the upper layers of sediments and leaf packages in the upper courses of streams under canopy. In winter the ammonium emerging from the sediment is not immediately incorporated in plant tissue. However, the richness in oxygen will promote the conversion to nitrate. As a result the winter concentrations of both ammonium and nitrate are high (Hendiksen et al., 1984). In spring the concentrations are lowered by the uptake of the rapidly growing planktonic community or the vegetation. In running waters ammonium is being oxydized to nitrate during the course from upstream to downstream. When, in summer the oxygen concentration is low, the conversion of ammonium either slows down or produces nitrite (Helder and de Vries, 1983; Morris et al., 1985), which can be toxic for the biota. The process of denitrification to nitrogen gas, which is strictly anaerobic, will prevail in summer. Only under near anaerobic conditions the denitrifying bacteria are capable of producing incompletely reduced compounds like nitrous oxyde.

The effects of eutrophication.

Fresh water.

Eutrophication is not a new phenomenon only introduced by recent activities of mankind. Natural causes of enhanced availability of plant nutrients may comprise erosion of sediments or the introduction of animal droppings. Already in early days, the water of the River Rhine and its estuary was in some way eutrophicated, as Lauterborn concluded in 1918. In a more recent study, comparing plankton inventories in the Dutch part of the river throughout this century, Peelen (1975) indicated that the progressive introduction of nutrients did only result in an increase of biomass and not in a dramatically altered species composition. The surface water in the Rijnland area, and probably in the entire western part of The Netherlands is characterized by high concentrations of total phosphorous, total nitrogen and chlorophyll. The phytoplankton species composition, and especially the wide-spread massive occurrence of certain cyano-bacteria (blue-green algae) is indicative for the highly eutrophic state of these waters. In "boezem" waters blooms of the species *Microcystis aeruginosa*, and in "polder" waters of *Oscillatoria* and *Lyngbia* species are revealing eutrophication (Hoogheemraadschap van Rijnland, 1984).

Ditches are determining the Dutch landscape to a large extend. The total length of these man-made small waterways amounts to 300,000 to 400,000 km (RIN, 1979). In flat agricultural country they are necessary for drainage and regulation of ground water levels. The biological significance of the ditches used to be caused by the diversity and gradients of water chemistry (salinity, trophic condition and hardness) and the traditional way of maintenance (removing vegetation regularly). Each type of ditch had its characteristic biocoenosis. Since 1950, the progressive increase of nutrient addition firstly caused stimulated growth of vascular water plants, which calls for more frequent removal of vegetation to prevent blocking of water flow. In a later stadium eutrophication has led to declining community diversity. Regarding the vegetation, the type of ditch vastly over-grown with different species of duckweed (*Lemna* spp., *Spirodela polyrrhiza*, *Wolffia arrhiza* and *Azolla filicoides*) has become dominant. Community structures characteristic for oligotrophic and mesotrophic

conditions are no longer present. Even moderately eutrophic conditions are scarce. They only occur in places where there is an influx of clean water, mainly seepage. The water of most ditches has to be labeled as hypertrophic (Leentvaar, 1979). On the one hand, the excessive use of artificial fertilizer has to be held responsible for the deterioration of the draining system of farm land. Whereas on the other hand the commonly applied alteration of drainage levels enhanced the effects. In spring it is advantageous for agricultural management to lower the ground water level. Especially in dry summers, water shortage has to be prevented by letting in river water, usually containing large quantities of nutrients.

The sandy areas in the eastern parts of The Netherlands are characterized by low-land brooks fed by groundwater; a type of watercourse very rare in Europe. Originally these streams accommodated rare plant species and a very rich fauna (Moller Pillot, 1971). Recently large scale land consolidation projects reduced the number of these brooks ("normalisation"). However, relatively little enrichment with nutrients proved to alter especially the communities in the mid-region of these brooks toward a species poor non-distinctive type (Dean & Burlington, 1963). Due to intensive cultivation, almost all still existing Dutch low-land brooks were subject to this development since 1965 (De Molenaar, 1980).

The situation in the Dutch peat pools (moorland pools) is even worse. Caused by the lesser productivity (limited by other nutrients), the amounts of unused accumulating nitrogen will be bigger than the accumulation in ditches. Since 1980 the ammonium nitrogen content of the water increased from one to more than two milligrams per liter in several moorland pools (Van Dam & Beljaars, 1984). There are some indications that the increased ammonium concentrations will cause a species shift toward land-forming species (Roelofs et al., 1983). In lime deficient moorland pools, the effects of nitrogen loading are strongly related to the problems caused by acidification. Nitrification diminishes rapidly when acidity decreases from pH 6.4 to 5.1 (Focht & Verstraete, 1977). Recently performed laboratory experiments indicate that in sediments of peat pools nitrification stops entirely at a pH of 3.9 (Schuurkes, 1985). From these findings it can be concluded that acidification leads to the accumulation of ammonium. As to the observed development of a pioneer vegetation in the badly buffered moorland pools in the eastern part of the Dutch sandy areas (Van Dam &

Dobbe, 1982) no definite answer can be given about the direct cause; acidification or accumulation of ammonium. In the west of The Netherlands, surface waters are that strongly buffered that no such effects have occurred.

In general it can be stated that the shift in plant species due to eutrophication with phosphorous is much more elaborately documented (De Lyon & Roelofs, 1986) than the shift due to nitrogen eutrophication. Based on nitrophilic rating numbers, the entire Dutch endemic flora showed a relative decrease of species with a preference for conditions poor in nitrogen (table 1).

TABLE 1.

The nitrogen related relative change of endemic higher plant species since the beginning of this century (Molenaar, 1980).

groups of plants	nitrogen index	percentage of total	increased	unchanged	decreased
Indicators for conditions poor of N	1,2	22%	2%	18%	80%
Preference for conditions poor of N	3,4,5	39%	3%	43%	54%
Preference for (some) nitrogen	6,7	25%	4%	53%	43%
Indicators for conditions rich of N	8	10%	11%	43%	46%
Indicators for conditions extremely rich of nitrogen	9	4%	15%	64%	21%

The marine environment

From the beginning of this century the supply of phosphorous and nitrogen from the rivers to the North Sea increased strongly, which resulted in

elevated concentrations along the Dutch coast. In the central part of the North Sea hardly any influence is noticeable. One of the frequently observed effects of nutrient loading is the occurrence of low oxygen concentrations caused by the aerobic decomposition of excessive amounts of organic substrates formed by an increase in primary production. In the Dutch Wadden Sea occasionally oxygen depletion is observed. The major impact, however, was noticed in the German Bight during the summer of 1982. An area of more than 15,000 square kilometers was seriously depleted of oxygen to an extent that fish and bottom dwelling organisms were killed. Some evidence exists that these events seldomly occurred before the sharp increase of eutrophication. Another effect is the shift in the dominance of phytoplankton species from diatoms to flagellates. The last few years this phenomenon can be clearly observed in the blooming of the species *Pheocystis*. A collapsing bloom of this species may induce the formation of large quantities of foam on the beaches. The nutritional enrichment of coastal water also induces more positive effects. The moderate increase of primary production in the Dutch Wadden Sea resulted in an increase of production and biomass of some worm species and shellfish. In the last decades shrimp fisheries produced more catch. As a consequence of better food conditions the growth rate of plaice and sole and the reproduction of plaice increased (Bannister, 1978).

The possibilities for control of eutrophication.

Until recently measures for counteracting eutrophication were focussed on a reduction of the amount of phosphorus entering the surface water. The Dutch government developed a plan to force back phosphorous load to one gram P per square meter per year (Fosfatennota, 1979). This plan consisted of replacing phosphates in detergents by other products, and removing phosphate in sewage treatment plants. The realisation of these measures did not progress as planned. On the one hand this is caused by the high costs involved in treatment and on the other, by the understanding that these measures will not lead to the expected improvement of water quality (Hoogheemraadschap van Rijnland, 1984). Other sources of phosphorous, such as diffuse fertilization in agriculture and the inflow with rivers will

still contribute to eutrophication. Furthermore, the problems related to eutrophication can not solely be attributed to excessive amounts of phosphorous. As has been mentioned previously, a large part of the Dutch surface water still suffers from nitrogen limited plant growth. Our incapacity to deal with the problem recently resulted in a more integral approach. Emphasis explicitly has to be put on the simultaneous suppression of all kinds of nutrient pollution of all sources. In addition to measures against well defined point sources, the only real solution of the nitrogen problem includes measures against the diffuse introduction of biologically available nitrogen from the atmosphere (dry and wet deposition of NOx compounds) and from agricultural activities.

The latter requires the acceptance of a totally different way of handling natural (manure) and artificial fertilizers. Agricultural management should aim at a maximum effective utilization of the administered nutrients. This will reduce the run-off, wash-out and evaporation of both phosphorous and nitrogen compounds. Measures in this direction include; 1) regulation of the period of the year manure may be applied, 2) ploughing after application of manure, 3) the applied dosage of manure and artificial fertilizer, or the combination thereof should be in relation to crop demand, 4) The growth season should be lengthened by combined cultivation, 5) surplus manure should be transported to areas with a shortage in organic fertilizer, 6) excessive production milk and live-stock should be diminished to more appropriate proportions, which also reduces the production of manure, and 7) the regulation of the ground water level should be adapted to minimize run-off and wash-out of nutrients ("Commissie uitrijverbod dierlijke meststoffen", 1986; Aart et al., 1986).

The necessity for the customary inlet of strongly polluted water of the rivers in summer can be reduced by preventing the extreme lowering of winter and spring levels in agricultural areas, and allowing for some aridity damage to occur.

The reduction of dry and wet atmospheric deposition of nitrogen compounds can be found in reducing or optimizing the combustion of fuel.

A number of possible measures can be found in treatment of the symptoms of eutrophication. The natural biological purification function of nutrient enriched water bodies can be enhanced by increasing the residence time of

the water (increasing the volume or compartmentalization of ditches), and by the creation of reed grown swamp like areas.

At this moment measures for reducing the eutrophication of the marine environment can only be found in decreasing the load of nitrogen and phosphorous in the inflowing water of the rivers (Lindeboom, 1986). In other words; pollution abatement has to start at the source. Especially for the removal of excess nitrogen compounds it has recently been proposed to use estuarine environments as large-scale treatment plants (e.g. the Ems-Dollard estuary). To what extend this proposal is practically feasible and to what extend nitrogen will be eliminated is an open question. Considering the fact that a large part of the estuary will suffer from the extra nitrogen load renders this management option undesirable.

Background levels and a proposal for target nitrogen values.

As a logical consequence of the processes underlying the nitrogen cycle, the assessment of the no effect level of eutrophication for nitrate only would be meaningless and useless. Depending on environmental conditions, all nitrogen will continuously be converted from one form in the other. Furthermore, the availability of oxygen, which is necessary for the production of nitrate, is inversely related to the progression of eutrophication. In oligotrophic surface water the nitrate formed by nitrification will immediately be incorporated in plant tissue. Therefore, the reduction of nitrogen eutrophication will be better served by producing a target value for total, biologically active nitrogen ($\text{NO}_2 + \text{NO}_3 + \text{NH}_4 + \text{organic N}$). The target value is bound to be related to the values originally observed in surface waters before eutrophication effects occurred. From figure 1 (Lindeboom, 1986) it can be concluded that a sharp increase of eutrophication took place since the end of World War II. Historical data on the nitrogen content of different types of water are very scarce. Mostly the analytical methods are not revealed, and analysis of all nitrogen compounds of interest are seldomly performed. In table 2 some data on different types of water are presented.

For the River Rhine the RIWA (1980) gives a nitrate value of 0.7 mgN/l for the period between 1946 and 1950. In 1979 total nitrogen amounts to 5.8

mgN/l, of which 4 mgN/l consists of nitrate. Assuming comparable ratios between the individual components, the total nitrogen concentration of the Rhine water was about 1 mgN/l in 1946-50. Fransz (1985) stated that total nitrogen increased by a factor 6 from 1930 to 1980, and again this leads to about 1 mgN/l in contrast to the 1.8 mgN/l Zijlstra (1980) claims for the

TABLE 2. Historical data.

Type of water	Year	N-NO ₂	N-NO ₃	N-NH ₄	N-(org + NH ₄)	Reference
averaged values in mgN/l						
Lake Braassem	1941-42	0.05 (0-0.12)	0.16 (.05-4.5)	0.78 (0-1.5)	--	Gruyter et al., 1950
Oude Rijn	1941-42	0.09 (0-0.15)	1.1 (0.5-2.3)	0.6 (0-1.2)	--	" " " "
13 Lakes NW Overijssel	mei '60	<0.01	<0.01	0.11 s=0.04	0.68 s=0.27	Leentvaar, 1965
	juli '60	<0.01	<0.01	0.21 s=0.07	0.52 s=0.24	
	oct '60	<0.01	<0.01	0.23 s=0.15	0.72 s=0.35	
5 Dune lakes 5x12 monthly observations	1963	<0.01	<0.01	0.24 s=0.24	0.67 s=0.38	Leentvaar, 1967
22 Fens	1960	<0.01	<0.01	0.61 s=0.73	1.45 s=2.05	Van der Voo, 1973
River Rhine	1874	--	0.34	0.15	--	Zijlstra, 1980
	1930	--	0.7	1.1	--	Zijlstra, 1980
	1946-50	--	0.7	--	--	RIWA, 1980
	1979	0.1	4.0	0.7	1.7	RIWA, 1980
River Meuse	1953	----0.68----		0.62	--	data RIZA
North Sea coastal area	1930	--	-----0.1----		--	Fransz, 1985

1930 concentrations of combined nitrate and ammonium. Recently (1986) Lindeboom produced a figure (fig. 1) revealing historical data on the combined nitrate and ammonium load per second of the River Rhine. Taking an average flow of 2,000 cubic metres per second and assuming no significant contribution of nitrite and organic nitrogen, the 1930 load value of 2 kg N per second also leads to a concentration of about 1 mgN/l.

Considering all data available, the target value for total, biologically active nitrogen must be equal to or slightly smaller than 1 mgN/l. A target value for nitrate only can not be given, however, the unpolluted value for nitrate will be less than 0.01 mgN/l for waters in the eastern part of The Netherlands. Since all major surface waters (lakes and ditches) in the west are more or less flushed with water from the rivers, a target nitrate value of about 0.4 mgN/l seems appropriate. The target for nitrogen nutrients in the coastal marine environment can be set on 0.1 mgN/l.

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Figure 1. The load of P and N in the River Rhine from 1930 to 1980
(after Lindeboom 1986).

