Environmental Health Criteria 5

NITRATES, NITRITES, AND
N-NITROSO COMPOUNDS

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While every effort has been made to present information in the criteria documents as accurately as possible without unduly delaying their publication, mistakes might have occurred and are likely to occur in the future. In the interest of all users of the environmental health criteria documents, readers are kindly requested to communicate any errors found to the Division of Environmental Health, World Health Organization, Geneva, Switzerland, in order that they may be included in corrigenda which will appear in subsequent volumes.

In addition, experts in any particular field dealt with in the criteria documents are kindly requested to make available to the WHO Secretariat any important published information that may have inadvertently been omitted and which may change the evaluation of health risks from exposure to the environmental agent under examination, so that the information may be considered in the event of updating and re-evaluating the conclusions contained in the criteria documents.

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Lyons, France, 16–21 February 1976

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List of Abbreviations

DMN	*N*-methyl-*N*-nitrosomethanamine (*N*-nitrosodimethylamine, dimethylnitrosamine)
DEN	*N*-ethyl-*N*-nitrosoethanamine (*N*-nitrosodiethylamine, diethylnitrosamine)
DMA	*N*-methylmethanamine (dimethylamine)
DEA	*N*-ethylethanamine (diethylamine)
TMA	*N,N*-dimethylmethanamine (trimethylamine)
TMAO	trimethyloxamine (trimethylamine oxide)

ENVIRONMENTAL HEALTH CRITERIA FOR NITRATES, NITRITES, AND N-NITROSO COMPOUNDS

A WHO Task Group on Environmental Health Criteria for Nitrates, Nitrites and *N*-nitroso compounds met in Lyons from 16 to 20 February 1976. Dr Higginson, Director of the International Agency for Research on Cancer opened the meeting on behalf of the Director-General. The Task Group reviewed and amended the second draft of the criteria document and made an evaluation of the health risks from exposure to these compounds.

The preparation of the first draft of the criteria document was based on national reviews of health effects research on nitrates, nitrites, and *N*-nitroso compounds received from the national focal points for colla- boration in the WHO Environmental Health Criteria Programme in Bulgaria, Canada, Czechoslovakia, the Federal Republic of Germany, Netherlands, New Zealand, Poland, the United Kingdom, the USA, and the USSR. Dr I. C. Munro, Toxicological Research Division, Health Protection Branch of the Department of National Health and Welfare, Ottawa, Ontario, Canada, prepared both the first draft and the second draft which took into account the comments received from the national focal points in Bulgaria, Canada, Czechoslovakia, Finland, Japan, New Zealand, Poland, Sweden, USA, and the USSR; from the United Nations Industrial Development Organization (UNIDO), the Food and Agriculture Organization of the United Nations (FAO), the United Nations Educational, Scientific and Cultural Organization (UNESCO), the International Atomic Energy Agency (IAEA), the Health Protection Directorate of the Commission of the European Communities (CEC), and from the International Federation of Pharmaceutical Manufacturers' Associations (IFPMA).

At the request of the Secretariat, comments were also received from Dr S. Oden, Agricultural College, Department of Soil Science, Division of Ecochemistry, Uppsala, Sweden.

The collaboration of these national institutions, international orga- nizations and individual experts is gratefully acknowledged. Without their assistance this document could not have been completed. The collaboration of the International Agency for Research on Cancer in the preparation of the document and in acting as host to the Task Group is also greatly appreciated.

The Secretariat wishes to thank Mr A. W. Kenny, Department of the

Environment, London, England and Mr D. A. H. Price, Chorley Wood, Herts, England for their advice in the preparation of some sections of the document and Dr Munro for his help in the final phases of editing.

This document is based primarily on national contributions and on original publications listed in the reference section. In addition, some recent publications reviewing the environmental health aspects of nitrates, nitrites and N-nitroso compounds have been used. These include reviews and symposia by the US National Academy of Sciences, Washington, DC (Committee on Nitrate Accumulation, 1972), the US Department of Health, Education and Welfare (1970), the US Environmental Protection Agency,[a] the International Agency for Research on Cancer (Bogovski & Walker, 1974; Bogovski et al., 1972a; Walker et al., 1970), Druckrey et al. (1967), Lee (1970a), Magee & Barnes (1967), Magee et al. (1976), Montesano & Bartch (1976), and Sen (1974).

Details concerning the WHO Environmental Health Criteria Programme including the definition of some terms frequently used in the documents may be found in the general introduction to the Environmental Health Criteria Programme published together with the environmental health criteria document on mercury (Environmental Health Criteria 1 — Mercury, World Health Organization, Geneva, 1976).

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[a] US Environmental Protection Agency (1976) Scientific and technical assessment report on nitrosamines. Preprint of document submitted for publication in the STAR series, Washington DC, Office of Reserch and Development, 210 pp.

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1. SUMMARY AND RECOMMENDATIONS FOR FURTHER STUDIES

1.1 Summary

1.1.1 Analytical methods

1.1.1.1 *Nitrates and nitrites*

The Task Group recognized that interpretation of the results of analyses of nitrates and nitrites in environmental and biological media would vary according to the analytical methods employed (e.g. spectrophotometry, spectrofluorimetry, nitrate specific electrode) and that this would make meaningful comparisons of much data in the literature difficult.

It was considered, however, that reported figures for water and for meat products could be compared as far as the assessment of health hazards was concerned. The Group also noted that, although the principle underlying a particular method of analysis may well be the same for a variety of substrates, difficulties usually arise during the sampling, extraction, and clean-up procedures which vary in complexity according to the nature of the substrate being analysed.

1.1.1.2 N-*nitroso compounds*

The detection and estimation of volatile N-nitroso compounds is complicated by the following basic issues: they are likely to be present in environmental media in concentrations of only 1 part in 10^9 parts; they occur in a complex matrix in food and biological samples many components of which will contain nitrogen and will react in a similar manner chemically; they must be isolated from this matrix in a form that permits their estimation and unequivocal identification. Whilst removal from the matrix is easy in the case of N-nitroso compounds of low molecular weight because they are steam-volatile, this approach cannot be used for non-volatile N-nitroso compounds. Analysis of these compounds has received scant attention so far, although some work on clean-up procedures exists, and the use of the liquid chromatography technique is now under investigation following its successful application to the separation of model mixtures of N-nitroso-N-alkyl ureas and the analogous urethanes.

Irrespective of the isolation techniques used, the quantitative determination of N-nitroso compounds requires a concomitant positive identification of the molecular species determined. For this reason, the preferred method of analysis, gas-liquid chromatography allied to a nitrogen-sensitive detector, must be linked to high-resolution mass

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spectrometry to confirm the presence of *N*-nitroso compounds. Results should be considered positive only when or if mass-spectroscopic techniques have confirmed their unequivocal presence.

1.1.2 Source and occurrence in the environment

1.1.2.1 *Nitrates and nitrites*

Nitrates are present naturally in soils, waters, all plant materials, and in meats. They are also found in small concentrations ($1-40$ $\mu g/m^3$) in air as a result of air pollution. Levels in cultivated soils and thus, levels in water, (which normally do not exceed 10 mg/litre) may be increased by the use of commercial nitrogenous fertilizers and by the return of wastes, derived from animal husbandry or other sources, to the soil. Nitrate contents of crops are influenced by the plant species, by genetic and environmental factors, and by agricultural management practices. In certain crops the levels may be very high (1000 mg/kg or more).

Nitrites are formed in nature by the action of nitrifying bacteria as an intermediate stage in the formation of nitrates, but concentrations in plants and water are usually very low. However, microbiological conversion of nitrate to nitrite may occur during the storage of fresh vegetables, particularly at room temperature, when nitrite concentrations may rise to exceptionally high levels (about 3600 mg/kg dry weight). Both nitrates and nitrites are widely used in the production and preservation of cured meat products and of some fish. Such uses, which are controlled by law in many countries, are considered vital for the prevention of botulism caused by the growth of the toxin-producing strains of *Clostridium botulinum* that are sometimes present in raw meat and that may persist in cooked meats. The weekly intake of nitrates by a member of the general population in England or in the USA has been roughly estimated to average about 400–500 mg but these figures cannot be applied generally because of variations in feeding habits and in the nitrate concentrations in food and water.

1.1.2.2 N-*nitroso compounds*

Low concentrations of *N*-nitroso compounds have been detected in air, water, and food, notably in nitrite-treated meat products and certain fish products. In most cases, the concentrations found in food have been in the $\mu g/kg$ range. No effective estimate of general population exposure to *N*-nitroso compounds can be made on the basis of these limited data. *N*-methyl-*N*-nitrosomethanamine (*N*-nitrosodimethylamine, DMN) has been detected in urban air samples and the presence of *N*-nitroso compounds, tentatively identified as *N*-nitroso derivatives of some

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pesticides, has been reported in samples from water treatment plants and river water in the USA.

The *in vivo* formation of N-nitroso compounds from nitrates or nitrites and amines or amides has been demonstrated in experimental animals and in one case in man.

1.1.3 Metabolism

1.1.3.1 *Nitrates and nitrites*

In normal healthy individuals, nitrates and nitrites are rapidly absorbed from the gastro-intestinal tract. Absorbed nitrite reacts with haemoglobin to form methaemoglobin which, in adults, is rapidly converted to oxyhaemoglobin by reducing systems such as NADH-methaemoglobin reductase. In infants up to three months old and in very young animals this enzyme system is not completely developed. Under these conditions, the methaemoglobin formed may increase in the body resulting in a characteristic clinical condition (methaemoglo-binaemia). Microorganisms present in the food and gastrointestinal tract of very young infants may convert nitrates to nitrites and thus exacerbate the problem in this age group. In healthy individuals, absorbed nitrates are rapidly excreted by the kidneys.

1.1.3.2 N-*nitroso compounds*

Published information on the absorption, metabolism, and elimi-nation of N-nitroso compounds is limited. In cases where experimental animal data are available, they demonstrate that N-nitroso compounds are rapidly absorbed from the gastrointestinal tract and that their biological half-time appears to be less than 24 h. A part of some compounds may be excreted unchanged via the kidney, or even exhaled, but the greater part is metabollically transformed (hydroxylation, chain shortening, ring-opening etc.) and several metabolites of N-nitroso compounds have been identified in urine. Significant amounts of some compounds such as DMN may be degraded completely and the resulting carbon dioxide exhaled. The extent of such degradation varies depending on the structure of the compound and the animal species involved.

1.1.4 Experimental studies in animals

1.1.4.1 *Nitrates and nitrites*

The major effect of nitrates and nitrites is the induction of methaemo-globinaemia, mostly readily observed in very young animals. Most experimental work has been connected with this problem although embryotoxic effects resulting in prenatal mortality, resorptions, and

decreased birthweights have been noted in rat pups whose mothers received drinking water containing sodium nitrite. In adult rats given drinking water containing nitrite for 24 months, methaemoglobin levels were elevated but not to the point of producing overt toxic effects. Animal species studied appeared to be fairly resistant to the induction of methaemoglobinaemia by nitrites, since high doses were required to induce even minimal changes. However, very young animals have not been studied extensively or sufficiently. Nitrates and nitrites do not appear to be carcinogenic but nitrite mutagenicity has been demonstrated in several non-mammalian test systems.

1.1.4.2 N-*nitroso compounds*

In experimental animals, the most important biological actions of *N*-nitroso compounds are their carcinogenicity and teratogenicity.

The carcinogenic action of *N*-nitroso compounds in animals is known to occur in many different organs. In general, the routes of administration do not influence the localization of the tumours. However, both dose level and dose rate may affect the organ involved and the type of tumour produced. Specific structural changes in both dialkyl nitrosamines and cyclic nitrosamines affect their carcinogenicity. *N*-nitroso compounds have been shown to be transplacentally carcinogenic, when given to animals in the second part of gestation, irrespective of the route of administration. Carcinogenicity following the combined administration of amines or amides and nitrites to animals has also been reported indicating the *in vivo* formation of *N*-nitroso compounds.

The mutagenic action of nitrosamides, noted in test systems, differs from that of nitrosamines in that the first group of compounds has been found to be mutagenic in almost all test systems, whereas nitrosamines seem only to be active in systems where metabolic activation occurs.

Nitrosamines are known to have toxic and sometimes lethal effects on animal embryos, whereas nitrosamides cause malformations in several organs and systems.

1.1.5 Epidemiological studies

1.1.5.1 *Nitrates and nitrites*

Adults do not appear to be harmed directly by exposure to the prevailing concentrations of nitrates and nitrites in the environment, although some recent studies have indicated that nitrate aerosols in the ambient air may act as respiratory irritants. However infants and very young children are particularly susceptible to the induction of methaemoglobinaemia by nitrates and nitrites, ingested in water and food, and

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veral cases of illness and death have been reported. In most cases of methaemoglobinaemia, well-water containing high concentrations of nitrates had been used in the reconstitution of infant dried milk preparations. Most instances have been associated with water containing more than 90 mg per litre but a few cases of methaemoglobinaemia in infants have been associated with the consumption of water containing less than 50 mg per litre. Cases of methaemoglobinaemia in babies fed with spinach purée or carrot juice (both of which may contain very high levels of nitrates) have been reported, but there are too few data to establish dose-response relationships.

1.1.5.2 N-*nitroso compounds*

So far, correlations have not been established that link cancer in man with exposure to N-nitroso compounds or their precursors, but the possible role of N-nitroso compounds and in particular their *in vivo* formation in the development of nasopharyngeal, oesophageal, and stomach cancer has been suggested.

1.1.6 Evaluation of health risks

1.1.6.1 *Nitrates and nitrites*

Epidemiological and clinical studies on man have shown that the main toxic manifestation resulting from the ingestion of nitrates and nitrites is methaemoglobinaemia. This has been confirmed by experimental animal studies. On the basis of available data, the Task Group concluded that the prevailing concentrations of nitrates and nitrites in food and water did not constitute a health risk for adult members of the general population and older children, but that the risk may be higher for infants under 6 months of age and particularly under 3 months. For this reason, the Group recommended that infant dried milk preparations should be reconstituted with low-nitrate water (at least below 45 mg/litre) and that low-nitrate vegetables should be used in baby foods.

Also, the use of nitrates and nitrites as food additives should be reduced to the minimum, and avoided in fresh meat and fish. Nitrate levels in public water supplies should comply with the tentative limit of 45 mg/litre recommended by the 1971 International Standards for Drinking Water.

1.1.6.2 N-*nitroso compounds*

Although the precursors of N-nitroso compounds (nitrites, amines, and amides) are known to be widely distributed in various environmental media, information concerning N-nitroso compounds is limited. However, they are known to be present in certain foods and experimental

animal studies have shown that they are formed in the body from
variety of precursors. This may also occur in man.

N-nitroso compounds are carcinogenic in a wide range of anima.
species, most are mutagenic in test systems, and some have been shown
to be teratogenic in animals.

Although there is no epidemiological or clinical evidence at present, it
is highly probable that these compounds may also be carcinogenic in
man. A quantitative estimation of the carcinogenic risk to man
associated with different levels of exposure is not possible, at this time,
because of inadequate data. For these reasons, exposure to N-nitroso
compounds and their precursors, (nitrites, amines, and amides) should
be kept as low as practically achievable.

1.2   Recommendations for Further Studies

1.2.1   Analytical problems

1.2.1.1   *Nitrates and nitrites*

The major need is for standardization of analytical methods. At
present, it is difficult to compare the studies reported by one laboratory
with those reported by another. While in many instances the principle
underlying the determination is the same for many of the studies
reported, the large variety of substrates containing nitrates and nitrites
gives rise to difficulties with respect to sampling, extraction, and clean-
up procedures. Further efforts are needed to standardize these analytical
procedures on an international basis. To this end, the efforts of
international and regional groups should be supported.

1.2.1.2   N-*nitroso compounds*

The principal problem associated with the determination of N-nitroso
compounds in food and other environmental media results from
interference by other components of the substrates. At present, positive
identification of N-nitroso compounds can be made only by mass
spectroscopie techniques. Since such techniques are expensive and not
generally available, alternative methods are required. In addition,
methods for the detection and determination of nonvolatile N-nitroso
compounds should be developed further.

1.2.2   Sources and levels in the environment

1.2.2.1   *Nitrates and nitrites*

Research should be undertaken to find acceptable substitutes for

nitrates and nitrites in the preservation of certain foods such as canned meats.

National surveys of nitrate levels in soils, water, plant materials, foods, especially meat and milk products, and air are required together with quantitative data concerning other factors considered to have an effect on these levels. Similar information on nitrite levels is required with particular reference to foods and to areas where significant microbiological reduction of nitrates is likely.

It is important that levels determined in survey work of this nature should be reported on the basis of standardized analytical methods to facilitate the eventual comparison of data from all sources. National authorities should be encouraged to publish survey data or to communicate them to the World Health Organization.

1.2.2.2   N-*nitroso compounds*

National surveys of food, air, and water for the presence of volatile and, where possible, nonvolatile N-nitroso compounds are required and any results reported should be confirmed by mass spectroscopy. More studies are needed on the chemical conditions under which N-nitroso compounds are formed (e.g. in mixtures of nitrites and amines or amides). The use of ascorbic acid for the prevention of nitrosamine formation and the inhibitory or catalytic effect of food constituents on the formation of N-nitroso compounds also require studies. The role of oxides of nitrogen as possible nitrosating agents should be investigated in relation to the occurrence of N-nitroso compounds in the environment (e.g. in the ambient and workroom air).

1.2.3   Metabolism

1.2.3.1   *Nitrates and nitrites*

Further work on the influence of ascorbic acid and other ingredients of the stomach contents on the metabolism of nitrates and nitrites is required. The treatment of infant dried milk formulae with ascorbic acid or by the introduction of *Lactobacilli* to prevent nitrate reduction should also be studied.

Other areas requiring investigation include: the influence of gastro-enteric disease on the development of methaemoglobinaemia; the influence of the total gut flora on nitrate metabolism *in vivo*; the relationship between ingested nitrate and salivary nitrate and nitrite levels.

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1.2.3.2 N-*nitroso compounds*

More knowledge should be gained on the *in vivo* formation of *N*-nitroso compounds in man and the factors involved. Studies comparing the metabolism of *N*-nitroso compounds in experimental animals and in man are considered to be of the greatest importance.

1.2.4 Experimental studies

Further research on the biological action of *N*-nitroso compounds should concentrate on dose-response relationships especially at low levels, and on their combined effects with other carcinogens, and environmental pollutants. The influence of nutritional factors on the carcinogenicity of *N*-nitroso compounds should be studied in more detail.

More inhalation studies are necessary to assess the importance of the recently reported occurrence of *N*-nitroso compounds in air and further research is needed on the quantitative aspects of the mutagenic activity of *N*-nitroso compounds and its possible significance for man.

1.2.5 Epidemiological and clinical studies

1.2.5.1 *Nitrates and nitrites*

With respect to the adverse effects of nitrates and nitrites on infants, there is a need to investigate the relationship between methaemoglobinaemia and sudden infant death and to make further studies on the role of gastroenteritis in increasing infant susceptibility to nitrate poisoning. The role of acidified milk preparations and *Lactobacilli* in protecting infants against methaemoglobinaemia, and the possible protective role of ascorbic acid fortification of infant milk preparations should also be elaborated.

1.2.5.2 N-*nitroso compounds*

Prospective and retrospective epidemiological studies in man, exposed to *N*-nitroso compounds, are needed. Efforts should be made to determine whether cancers, that are peculiar to special areas of the world, might be due to exposure to *N*-nitroso compounds. Chemical analyses of the environment for *N*-nitroso compounds and their precursors should be carried out in conjunction with these epidemiological studies.

2. CHEMISTRY AND ANALYTICAL METHODS

2.1 Chemical Properties and Reactions

2.1.1 Nitrates and nitrites

The nitrate ion (NO_3^-) is the conjugate base of nitric acid (HNO_3). Nitric acid is a strong acid ($pK_a = -1.37$) which dissociates in water yielding nitrate ions and hydroxonium ions (H_3O^+). Salts of nitric acid (nitrates) are readily soluble in water with the exception of the basic nitrates of mercury and bismuth.

The nitrite ion is the conjugate base of nitrous acid (HNO_2) which is a weak acid ($pk_a = 3.37$) and exists only in cold dilute aqueous solution because it decomposes readily to give water and dinitrogen trioxide (N_2O_3) or nitric acid, nitric oxide (NO), and water. Salts of nitrous acid (nitrites) are much more stable than the acid itself and are readily soluble in water with the exception of silver nitrite.

In the environment (e.g. surface waters, soil) both nitrite and nitrate ions can be formed from the ammonium ion (NH_4^+) in a two step biological oxidation (nitrification) process:

$$2\,NH_4^+ + 2\,OH^- + 3O_2 \rightleftarrows 2\,NO_2^- + 2H^+ + 4\,H_2O \qquad (1)$$

$$2\,NO_2^- + O_2 \rightleftarrows 2\,NO_3^- \qquad (2)$$
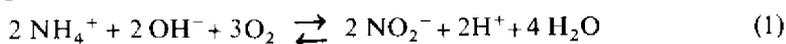
These two reactions are mediated by different microorganisms: reaction (1) by an aerobic chemolithotroph *Nitrosomonas*; reaction (2) by *Nitrobacter* which obtains almost all its energy from the oxidation of nitrites.

Higher plants assimilate nitrite from the soil by (*a*) reduction of nitrate to nitrite which is catalysed by nitrate reductase (NADPH) (1.6.6.3), and (*b*) reduction of nitrite to ammonia catalysed by nitrite reductase (1.7.99.3). Bacteria of many kinds can also reduce nitrate to nitrite. However, because nitrite is easily oxidised to nitrate the concentration of nitrites in environmental media such as surface waters is usually very low (about 1 mg/litre) even when the nitrate concentration is high (50–100 mg/litre).

These biochemical reactions are a part of the nitrogen cycle which is further discussed in section 3.1.

2.1.2 *N*-nitroso compounds

N-nitroso compounds have a general structure

$$\begin{matrix} R_1 \\ R_2 \end{matrix} \Big\rangle \ N\text{---}N{=}O$$

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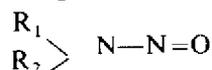
They can be divided into two classes with different chemical properties (Druckrey et al., 1967; Fridman et al., 1971):

(1) nitrosamines where R_1 and R_2 are alkyl or aryl groups; and
(2) nitrosamides where R_1 is an alkyl or aryl group, and R_2 is an acyl group.

Nitrosamines are generally stable compounds that only slowly decompose in the light or in aqueous acid solutions.

In contrast, nitrosamides are much less stable in aqueous acids and unstable in basic solutions. Examples of nitrosamides are N-alkyl-N-nitrosoureas (3) and N-alkyl-N-nitrosourethanes (4).

$$R\!-\!N\!-\!C\!-\!NH_2 \tag{3}$$
$$\quad\ |\quad\ \|$$
$$\quad\ NO\ \ O$$

$$R\!-\!N\!-\!C\!-\!OC_2H_5 \tag{4}$$
$$\quad\ |\quad\ \|$$
$$\quad\ NO\ \ O$$

The physical properties of N-nitroso compounds vary widely depending on the substituent groups. Some like N-methyl-N-nitrosomethanamine (dimethylnitrosamine, DMN) are oily liquids miscible with polar solvents. Some are solids e.g. N-nitroso-N-phenylbenzenamine (diphenylnitrosamine) and are only slightly soluble in ethanol and practically insoluble in water. The lipid/water partition coefficients vary widely. Nitrosamines show ultraviolet absorption peaks in water at 230–240 nm and 330–350 nm. For nitrosamides, the long-wavelength absorption peak in water is at 390–420 nm. Some N-nitroso compounds are volatile (Mirvish, 1975, 1976; Sen, 1974). Physical properties of N-nitroso compounds have been listed by Druckrey et al. (1967), Fieser & Fieser (1967), and Weast (1976).

Nitrosamines may react by "transnitrosation" i.e. as nitrosating agents to nucleophilic[a] species (Buglass et al., 1974). This reaction may have important biological implications.

2.1.3 Formation of N-nitroso compounds *in vitro*

The formation of N-nitroso compounds from amines and nitrites has been reviewed by Mirvish (1975), Sander (1971a, 1971b), and Sander & Schweinesberg (1972).

For example, for N-methylmethanamine (dimethylamine) (DMA) and

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[a]i.e. electron-rich.

sodium nitrite in dilute hydrochloric acid solutions, nitrositation is considered to proceed as follows (Mirvish, 1970):

$$NaNO_2 + HCl \rightleftarrows HNO_2 + NaCl \tag{5}$$

$$2HNO_2 \rightleftarrows N_2O_3 + H_2O \tag{6}$$

$$(CH_3)_2NH + N_2O_3 \rightleftarrows (CH_3)_2HN-NO + NO_2^- \tag{7}$$
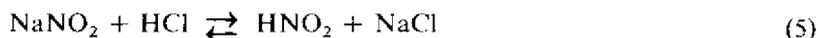
The reaction rate depends on the concentration of nonionized amine and nitrous acid. At pH > 1, the main nitrosating agent is dinitrogen trioxide which is formed reversibly from 2 molecules of nitrous acid. The rate of reaction (7) is proportional to the concentration of dinitrogen trioxide, $[N_2O_3]$, and hence to the square of nitrous acid concentration, $[HNO_2]^2$, i.e.

$$\text{rate (7)} = k_1[N_2O_3][HNO_2]^2 \tag{8}$$

The concentrations of nonionized amine and of free nitrous acid vary with pH but k_1, is independent of pH. For practical purposes it is more convenient to rewrite equation (8) in terms of the total concentrations of nitrite and DMA i.e.

$$\text{rate (3)} = k_2 [\text{total amine}][\text{total nitrite}]^2 \tag{9}$$

where k_2 depends on pH; k_2 and the reaction rate show maximum values at pH = 3.4 corresponding to the strength of nitrous acid (pk_a = 3.37). The reaction rate decreases tenfold for each 1-unit increase in pH above pH = 3.4. Below this pH level, the nitrite is almost completely converted to nitrous acid. The main effect of a further reduction in pH is a continuous drop in nonionized amine concentration, causing a decrease in the reaction rate. There is no sharp pH limit for nitrosation. It can occur slowly at a pH of 5 or even 6, as observed for DMA (Mirvish, 1970).

The nitrosation of amides, such as N-alkylureas and N-alkylurethanes proceeds rapidly (Challis & Challis, 1970; Mirvish, 1971; Sander & Burkle, 1969). In this case the nitrosating agent is probably the nitracidium ion $(H_2NO_2)^+$:

$$2HNO_2 \rightleftarrows (H_2NO_2)^+ + NO_2^- \tag{10}$$

or

$$HNO_2 + H^+ \rightleftarrows (H_2NO_2)^+ \tag{10a}$$

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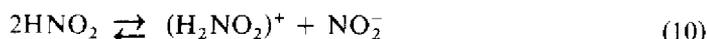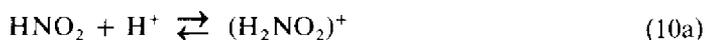
and nitrosation is accomplished by the following reaction:

$$RNH.COR' + (H_2NO_2)^+ \rightarrow RN(NO).COR' + H_2O + H^+ \qquad (12)$$
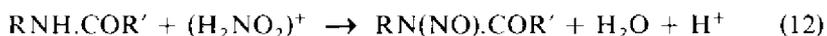
The reaction rate is again proportional to the concentrations of nonionized alkylurea and nitracidium ions the formation of which can be considered to proceed by equation (10a). Hence

$$\text{rate } (12) = k_3 [RNH.COR'][HNO_2][H^+] \qquad (13)$$

or

$$\text{rate } (12) = k_4 [\text{total amide}][\text{total nitrite}][H^+] \qquad (14)$$

The reaction rate, which increases about tenfold for each 1-unit drop in pH from 3 to 1, does not show a pH maximum; k_4 depends on the ionization of nitrite and, hence, on pH but it does not depend on the ionization of amides, which are only slightly ionized above $pH = 2$.

Tables giving the rate constants for 15 amines and 21 amides according to the above equations (Mirvish, 1975), indicate that the most rapidly nitrosated classes of compounds are the *N*-alkylureas, *N*-arylureas, *N*-alkylcarbamates, secondary aromatic amines, secondary amine piperazine, morpholine derivatives, and tertiary enamines.

It has been suggested that under mildly acidic conditions tertiary amines also react with nitrous acid to produce nitrosamines (Hein, 1963; Lijinsky, 1974; Lijinsky & Greenblatt, 1972; Lijinsky & Singer, 1974; Lijinsky et al., 1972b; Roberts & Caserio, 1964; Smith & Loeppky, 1967). Ender et al. (1967) studied the reaction between nitrites and various methylamines including: methanamine (monomethylamine); DMA; *N,N*-dimethylmethanamine (trimethylamine, TMA); and trimethyloxamine (trimethylamine oxide, TMAO); they found that DMN was produced in all cases. However, the rate of production was proportional to the amount of nitrite present and increased with decreasing pH values and increasing temperature. DMA was the most reactive followed by TMA. Small amounts of DMN were formed from DMA and sodium nitrite under very mild conditions (e.g. at 4°C). At pH $= 6.0$, 2 to 2.5 times more DMN was formed than at $pH = 6.5$. However, Malins et al. (1970), who failed to detect DMN formation at pH levels of 5.8–6.4 after heating an aqueous mixture of sodium nitrite and TMAO or DMA, found that trace amounts of DMN were detectable in reaction mixtures consisting of TMA at concentrations of 400–2000 mg/litre and sodium nitrite at 400 mg/litre.

Fiddler et al. (1972) demonstrated the formation of DMN from quaternary ammonium compounds and nitrite. The compounds studied included *N,N,N*-trimethylethaminium chloride (neurine chloride), 2-

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(acetyloxy)-N,N,N-trimethylethanaminium chloride (acetylcholine chloride), choline chloride, 1-carboxy-N,N,N-trimethylmethanaminium hydroxide (betaine), and 3-carboxy-2-hydroxy-N,N,N-trimethyl-1-propanaminium chloride (carnitine chloride).

Nitrites are present in various foods and in saliva (Tannenbaum et al., 1974) and can be formed in the infected bladder by bacterial reduction. They may also be present in the stomach of infants and of achlorhydric subjects where they are formed from nitrates, lower acidity allowing the growth of nitrate-reducing bacteria.

Secondary amines are widely distributed in foods and have been found in fish, eggs, rolls, biscuits, chocolate, soup cubes, meats, and potatoes (Heyns, 1973, Lijinsky & Epstein, 1970). Tobacco and tobacco smoke contain several secondary amines including pyrrolidine, DMA, and piperidine (Neurath, 1972). Some aliphatic and heterocyclic amines were identified in human blood and urine (Asatoor & Simenhoff, 1965; Perry et al., 1962). Other sources of secondary amines have been given by Sander et al. (1971).

Methylguanidine, a natural constituent of beef (Kapeller-Adler & Krael, 1930a) and shark, rayfish, and cod (Kapeller-Adler & Krael, 1930b), reacted with nitrite to produce N-methyl-N-nitrosourea and N-methyl-N-nitrosocyanamide (Mirvish, 1971). The amino acids l-proline, l-hydroxyproline, and N-methylglycine (sarcosine) were nitrosated 140–230 times more quickly than DMA at pH $= 2.2$ 2.5 (Mirvish et al., 1973a).

N-nitroso compounds formed from 22 natural compounds were listed by Mirvish (1975). In addition, nitrosation of N,N-bis (3 aminopropyl)-1, 4-butanediamine (spermine) and spermidine, two polyamines, was reported by Ferguson et al. (1974) and Hildrum et al. (1975).

2.1.4 The effects of other substances on the formation of N-nitroso compounds

Several substances have been shown to catalyse the formation of nitroso compounds from secondary amines and nitrite. Boyland & Walker (1974) and Fan & Tannenbaum (1973) noted that chloride, bromide, iodide, and thiocyanate catalysed the reaction while sulfate and perchlorate ions did not have any effect. The effects of thiocyanate have been studied more extensively; in its absence, the nitrosation of N-methylbenzenamine (N-methylaniline) and other secondary amines is at a maximum at pH $= 3$, but in its presence, the reactions proceed much more rapidly between pH levels of 1 and 2. Thiocyanate is present in amounts of 110 330 mg/litre in human saliva. It has been estimated that

the thiocyanate concentration in the stomach is 3 times higher in smokers than in nonsmokers.

Roller & Keefer (1974) reported a pronounced increase in the rate of formation of DMN from DMA and nitrite in the presence of certain carbonyl compounds and at a pH level higher than 3. Formaldehyde was the most effective catalyst and the effect was appreciable even at pH = 9. Challis & Bartlett (1975) reported that 3-[[3-(3,4-dihydroxy-phenyl)-1-oxo-2-propenyl]oxy]-1,4,5-trihydroxycyclohexanecarboxilic acid (chlorogenic acid), a constituent of coffee was a potent catalyst and in studies by Walker et al. (1975) 3,4,5-trihydroxybenzoic acid (gallic acid) catalysed the nitrosation of amines but only within a restricted pH range (around pH = 4).

On the other hand, Bogovski et al. (1972b) noted that tannins, which are present in many foods, competed with secondary amines for nitrite and thus led to a reduction in the amount of nitrosamine formed. Similarly Challis (1973) demonstrated the preferential nitrosation of phenols in the presence of amine to form p-nitrosophenols suggesting a scavenging effect of phenols at low pH.

Ascorbic acid inhibited the formation of DMN from oxytetracycline and nitrite and also from aminophenazone (aminopyrine) and nitrite (Mirvish et al., 1972, 1974). The same authors reported that gallic acid, the active ingredient in tannins, completely inhibited nitrosomorpholine formation from the parent amine and nitrite and that sodium sulfite had a similar blocking activity.

The inhibitory effects of ascorbic acid and other inhibitory agents on chemical nitrosation have recently been compared by Mirvish et al., 1975 and it would seem, at present, that ascorbic acid is the most effective and useful inhibitor of amine nitrosation.

2.2. Analytical Methods

2.2.1 Nitrates and nitrites

Methods for the determination of nitrates and nitrites in surface and waste waters have been reviewed by Marculescu (1971). The most suitable methods are colorimetric procedures using sodium salicylate for nitrates and 4-aminobenzenesulfonic acid (sulfanilic acid) and 1-naphthalenamine (1-naphthylamine) for nitrites.

A standard procedure for determining nitrates in plants (HMSO, 1973) is based upon the reduction of nitrates to ammonia which is removed by steam distillation and determined titrimetrically. The nitrate electrode has been used in the determination of nitrates in extracts of

soils and herbage, and drainage water in the United Kingdom (HMSO, 1974) and in the Federal Republic of Germany (Weil & Quentin, 1973). Results indicated that several extraction procedures applied to herbage gave higher values with the nitrate electrode than with the standard distillation procedure. For drainage waters, better agreement was obtained between the electrode and a spectrophotometric procedure involving, 3,4-xylenol.

A variety of methods is available for the determination of nitrates and nitrites in foods. A nitrate specific electrode for the electrochemical determination of nitrate in spinach suspensions was tested by Voogt (1969). Other anions present in the spinach did not have any direct influence on the precision of the results. Variations in nitrate activity due to variations in the ionic strength of the spinach extracts could be minimized by measuring the potential of the extract in a 1% sodium sulfate solution. The precision of the method was $\pm$ 2%. Kamm et al. (1965) developed a new method for the determination of nitrates and nitrites in foods that would accurately determine concentrations as low as 1 mg/kg. 1-Naphthylamine was diazotized by nitrite and coupled with excess amine to give 4-(1-naphthylazo)-1-naphthylamine which was measured spectrophotometrically. Nitrate was quantitatively reduced by passage through a cadmium column and determined as nitrite. Nitrite passed through the column unaltered; thus nitrate was determined by difference. Spectrophotometric and spectrofluorimetric methods for the determination of low levels of nitrite in cheese were developed by Rammel & Joerin (1972). The limits of detection for the two methods were 50 µg and 3.0 µg of nitrite-N respectively, per kg of cheese or milk products. A method to determine free and bound nitrite in meats was published by Mirna (1974). Free nitrite was determined with the Griess reagent whereas bound nitrite was liberated with Hg^{2+} in aqueous acetone solution prior to diazotization. Methods of analysis for nitrates and nitrites in several food products including meats, cured meats, dry cure mix of curing pickle, flours, and baby foods have been described (Horwitz, 1975) and adequate methods for the determination of nitrates and nitrites in urine and blood are also available (Shechter et al., 1972; Schneider & Yeary, 1973; Wegner, 1972).

2.2.2 *N*-nitroso compounds

The problems of estimating *N*-nitroso compounds in food and other environmental media were recently reviewed by Bogovski & Walker (1974), Bogovski et al. (1971a), Eisenbrand (1973), Fiddler (1975), Scanlan (1975), Sen (1974), and Walker et al., (1976). The analytical

process can be divided into three major steps: extraction and distillation from the specimen; purification; and qualitative and quantitative determination.

The main difficulties in such analyses arise from the fact that nitrosamines occur at very low concentrations and that they lack suitable characteristics for trace analysis. They also suffer from interference from other chemicals in the substrate which gives rise to a considerable number of false-positive reports of the presence of nitrosamines.

Most of the nitrosamines so far detected in foods are steam volatile. Many analytical methods take advantage of this fact and, in most of them, nitrosamines are isolated by distillation from an aqueous, acidic, or basic solution. Distillation from an acidic solution has the additional advantage of removing interfering amines. Howard et al. (1970) digested fish samples with methanolic potassium hydroxide before subjecting them to distillation. Telling et al. (1971) reported improved recoveries of nitrosamines by vacuum distillation. Other workers (Kröller, 1967; Sen et al., 1969a, 1972) preferred initial extraction of the nitrosamines with ether or methylene chloride prior to aqueous distillation. However, these techniques cannot be used for the analysis of nonvolatile nitrosamines such as nitrosoproline and nitrososarcosine.

Various column chromatographic procedures have been reported for the clean-up of nitrosamines isolated from foods and biological materials. These include ion-exchange (Alam et al., 1971a, 1971b; Sen et al., 1969a, 1969b) or basic alumina columns (Sen, 1970; Sen et al., 1970; Telling et al., 1972). These preliminary clean-ups have proved to be extremely useful in cases where nitrosamines were estimated by the conventional thin layer chromatography (TLC) and gas-liquid chromatography (GLC) methods but such clean-ups were thought to be unnecessary when a highly specific method such as high resolution gas-liquid chromatography–mass spectroscopy (GLC–MS), was used for the analysis.

Detection techniques can be divided into screening methods suitable for routine surveys, and confirmation techniques to be used if the results of the preliminary screening technique are positive. Combined high resolution GLC–MS is believed to be the only reliable confirmation technique available at the moment.

Eisenbrand & Preussman (1970) have described a colorimetric technique in which nitrosamines are cleaved to nitrosyl bromide and secondary amines, and the liberated NO^+ ion is measured colorimetrically after reacting with N-1-naphthalenyl-1,2-ethanediamine (N-(naphthyl-(1)-) ethylenediamine). The technique appears to be reliable and applicable to a wide variety of nitrosamines. The aminies formed

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after splitting may also be used to estimate nitrosamines through the formation of fluorescent 5-(dimethylamino)-1-naphthalenesulphonyl (dansyl) derivatives.

Various TCL methods have been used for the detection and semi-quantitative estimation of nitrosamines (Eisenbrand & Preussman, 1970; Kröller, 1967; Möhler & Mayrhofer, 1968; Sen & Dalpé, 1972; Sen et al., 1969a, 1973a; Yang & Brown, 1972). Most of these methods are based on the principle of splitting the nitrosamines by UV radiation into the parent secondary amines and nitrous acid, and subsequently detecting these breakdown products with 2,2-dihydroxy-1H-indene-1,3(2H)-dione (ninhydrin) N-phenylbenzeneamine (diphenylamine), and Griess reagent, respectively. In some methods, nitrosamines are reduced to hydrazines which are detected on TLC plates after the formation of suitable derivatives.

GLC offers a rapid and sensitive technique for the analysis of nitrosamines. In earlier work, the flame ionization detector was used but it was later abandoned because of the lack of sensitivity, and specifity. In more recent studies, various nitrogen-specific detectors have been used such as the alkaline-flame ionization detector (Fiddler et al., 1971; Howard et al., 1970; Kawabata, 1974), the Coulson electrolytic conductivity detector (Crosby et al., 1972; Issenberg & Tannenbaum, 1972; Panalaks et al., 1972; Rhoades & Johnson, 1970; Sen et al., 1972, 1973a), and the microcoulometric detector (Newall & Sisken, 1972). Each has some advantages and disadvantages, and the reader is advised to consult the original papers for further details. In one technique, nitrosamines were oxidized to the corresponding nitramines which were then detected by an electron capture detector (Althorpe et al., 1970; Castegnaro et al., 1972; Sen, 1970; Telling, 1972). Alliston et al. (1972) and Eisenbrand (1972) converted the nitrosamines to the parent amines from which the heptafluoro-butyryl derivatives were prepared and determined by electron capture detector.

Recently, Fine & Rufeh (1974) and Fine et al. (1974) have reported a new instrument which is specific to the N-nitroso functional group and is capable of detecting N-nitroso compounds in foodstuffs at the µg/kg level with little or no concentration or purification. In this technique, the N-nitroso compounds are cleaved at the N-NO bond in the presence of a specific catalyst, and the liberated NO is converted to excited nitrogen dioxide (NO_2^*) by reaction with ozone. As the excited nitrogen dioxide rapidly decays, it emits light in the near infrared region of the spectrum which can be detected and measured. The instrument can be coupled either to a GLC or a high pressure liquid chromatograph thus making it suitable for the analysis of both volatile and nonvolatile nitrosamines.

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The nonvolatile nitrosamines constitute a more varied group of compounds than the volatile nitrosamines and, as such, cause additional analytical problems. Nitrosoamino acids, such as nitrososarcosine, nitrosoproline, and nitroso-2-hydroxyproline may be analysed by conversion to volatile derivatives such as silyl ethers (Eisenbrand et al., 1975).

Methods have been proposed for the determination of total N-nitroso compounds, the general approach being to cleave the nitroso group and measure the nitric oxide formed. Fan & Tannenbaum (1971) eliminated the problem of nitrate interference by using long-wave ultraviolet irradiation (360 nm) to split the nitroso group. The released nitrite was diazotized and coupled to form a dyestuff before colorimetric estimation. The method was designed for automation. Eisenbrand & Preussmann (1970) used hydrobromic acid in a nonaqueous medium to split the nitroso group. A method for splitting the nitroso group which does not require any anhydrous medium has been proposed by Fine et al. (1976a).

3. SOURCES OF NITRATES, NITRITES AND N-NITROSO COMPOUNDS IN AIR, WATER, SOIL AND FOOD

3.1 Natural Occurrence

3.1.1 Nitrates and nitrites

Nitrates in soil and in surface and groundwater result from the natural decomposition by microorganisms of organic nitrogenous material such as the protein in plants, animals, and animal excreta. The ammonium ion formed is oxidized to nitrites and nitrates (section 2.1.1). Natural occurrence of nitrates and nitrites in the environment is a consequence of the nitrogen cycle (section 4.1) but normally nitrites are only found in very low concentrations.

3.1.2 N-nitroso compounds

Systematic studies on the natural occurrence of N-nitroso compounds have not been reported but a few studies show that these compounds may occur in certain microorganisms (Murthy et al., 1966; Vavra et al., 1960) and in one variety of mushroom (Hermann, 1960). At least one of these compounds, strephozotrin, is a potent carcinogen (Arison & Feudale, 1967; Sibay & Hayes, 1969). Other reports on the natural occurrence of diethylnitrosamine (DEN) in certain plants have still to be confirmed by modern analytical methods.

3.2 Sources Related to Man's Activities

3.2.1 Nitrates and nitrites

3.2.1.1 *Fertilizers*

Artificial fertilizers, a major source of environmental nitrates, may be composed of a variety of chemicals including ammonium, calcium, potassium, and sodium nitrates, and urea. The production of nitrogenous fertilizers in the world has increased in terms of N from 15.8 million tonnes in 1961/62–1965/66 to 42.3 million tonnes in 1974/75 (United Nations, 1976).

The fact that plants cannot use soil nitrogen completely is of great significance; nitrogen utilization may vary from 25 to 85% depending on the crop and on agricultural techniques. Thus, to obtain maximum production, a great excess of nitrogen fertilizer must be applied to the soil and the resulting nitrogen runoff will be substantially increased. For example, Kohl et al. (1971) showed that as much as 55–60% of the nitrogen input in the Sangamon River feeding Lake Decatur, IL, USA, was of fertilizer origin. Lee (1970b), Sawyer (1947), and Sylvester (1961), have all published data showing that nitrogen runoff is 3–10 times higher from fertilized areas than from unfertilized areas in the same region. However, analysis of stream waters did not show a clear relationship between the nitrate concentrations in British rivers and the amounts of fertilizers used on adjacent land (Tomlinson, 1970).

Brown & Smith (1967) observed that nitrogen fertilization tended to increase the nitrate content of vegetables and attempts have been made to correlate nitrogen application rates with the nitrate contents of lettuce, radish, and spinach. In studies in Bulgaria, Biočev & Počinkova (1972) noted that the nitrate levels in spinach increased when as little as 20 kg of nitrogen per ha was added to the soil. Schuphan (1969) observed that application of four times the normal amount of fertilizer resulted in considerably higher nitrate levels in spinach but that the nitrite levels remained low.

3.2.1.2 *Animal wastes*

Another major source of nitrates is farm animal wastes which contain large amounts of nitrogenous materials that may be converted into nitrates. The problem is more acute where farming is carried out intensively, a common practice in North America for both livestock and poultry. Since a 450 kg steer excretes about 43 kg of nitrogen per year, a 3200 head feedlot would produce 1400 tonnes annually on a relatively small area—an amount equivalent to about 260 000 people. Thus, such feedlots become "small area" sources of nitrogen runoff. Only 10% of

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these wastes is returned to cultivated land (Standford et al., 1969) and runoff studies demonstrate a considerable problem of environmental pollution. Nye (1973) reported Gilbertson et al. (1970), who found that the total nitrogen concentration in runoffs ranged from about 50 to over 5500 mg/litre. Animal husbandry, even when carried out on pastures or with the return of the animal wastes to cultivated land, may still impose problems. Adriano et al. (1971) concluded that wastes from a maximum of 7 8 cows could efficiently be used per hectare of farmland or pasture and that higher application rates might raise nitrate levels above 10 mg/litre in the subsoil waters.

3.2.1.3 *Municipal, industrial, and transport wastes*

Discharges of municipal and industrial wastes are concentrated sources of nitrogen compounds that are, to a large extent, released directly into surface waters. The amount of nitrogen in human wastes is estimated to be about 5 kg per person per year (Committee on Nitrate Accumulation, 1972). Even if treated, this waste will represent a heavy water pollution load since secondary treatment removes less than half of the nitrogen. Ammonium ions in the effluent of septic tanks may be rapidly converted to nitrate which may penetrate some distance from the tank. Sludge from treatment plants and septic tanks has also to be disposed of and represents another significant source of nitrogen pollution. Solid waste disposal practices, particularly sanitary landfills and dumps, may represent a source of water pollution by nitrogen compounds.

The nitrogen content of industrial wastes is highly variable; fuel and food processing industries and petroleum refineries may constitute important sources of nitrogen pollution. The nitrogen/BOD^a ratio of food processing plant wastes is about 0.05 while for animal processing wastes this ratio amounts to 0.5. (Committee on Nitrate Accumulation 1972). Oxides of nitrogen released into the atmosphere from man-made sources such as motor vehicles, fossil fuel combustion, and industrial processes amount to about 50 million tonnes per year on a global scale (Robinson & Robbins, 1972). A considerable proportion of this fixed nitrogen is eventually returned to the earth's surface as nitrate.

3.2.1.4 *Deliberate addition of nitrates and nitrites to food*

Nitrates and nitrites are widely used in the production of certain meat products and in the preservation of fish in some countries. Reasons for using these salts in food production have been reviewed by Ingram

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^{a}BOD = biological oxygen demand.

(1974). Nitrite is used in meat curing to obtain the characteristic pink colour and flavour of cured meat. While a nitrite content of less than 5 mg/kg of meat is sufficient to give a satisfactory colour for a limited period of time, up to 20 mg/kg may be necessary to give commercially adequate colour stability and about 50 mg/kg to produce the characteristic flavour. However, detailed experimental confirmation of these figures is lacking.

Curing meat gives an important degree of protection against botulism and may provide similar protection against other bacteria such as *Clostridium welchii* and staphylococci, although the importance of this has not yet been assessed. The question of how much nitrite is necessary to protect against botulism is very complex because of several associated factors.

The addition of nitrates and nitrites to meats, meat products, and cheese is governed by legislation in most countries, some of which also allow the addition of these salts to fish products.

3.2.2 *N*-nitroso compounds

3.2.2.1 *Food*

The formation of *N*-nitroso compounds from nitrites and amines during the storage and processing of food is discussed in section 4.2.2.

3.2.2.2 *Tobacco*

Nitrosonornicotine has been found in unburned smoking tobacco, chewing tobacco, and in snuff. The same compound has been identified in the mainstream smoke of a popular nonfilter cigarette in the USA (Hoffman et al., 1974).

3.2.2.3 *Industrial uses*

Although *N*-nitroso compounds do not appear to be extensively used at present, Magee (1972) reports that patent applications have been made in the UK for their use in the manufacture of dyestuffs, lubricating oils, explosives, insecticides, and fungicides. Some nitrosamines (nitrosodiphenylamine, *N-N*-dinitrosopentamethylenetetramine, polymerized *N*-nitroso 2,2,4-trimethyl-1,2-dihydroquinoline and *N*-methyl-*N*, 4-dinitrosoaniline) are used as organic accelerators and antioxidants in the production of rubber (Boyland et al., 1968). DMN has been used as an industrial solvent, as a nematocide, and in the synthesis of the rocket fuel 1,1-dimenthylhydrazine. There is some evidence that DMN might also be formed during the combustion of this rocket fuel (Simoneit & Burlingame, 1971). There are patents for the use of DMN as a solvent in

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the plastics and fibre industry, as an additive for lubricants, and to increase the dielectric constant in condensers (Daiber, 1966).

Industrial uses may result in the occurrence of N-nitroso compounds in the work environment and in industrial effluents. Fine et al. (1976b) reported point sources of air pollution by DMN in Baltimore, MD, and Belle, WV, in the USA. A factory using DMN as an intermediate was shown to be the source in Baltimore and was shut down; in Belle the source of DMN was an amine-manufacturing facility.

4. TRANSPORT AND TRANSFORMATION IN ENVIRON-MENTAL AND BIOLOGICAL MEDIA

4.1. Nitrogen Cycle

The continuous interchange between atmospheric and terrestrial nitrogen takes place along a number of different pathways including air, water, soil, microorganisms, plants, animals, and man. This transfer and transformation of nitrogen is referred to as the nitrogen cycle (Fig. 1).

The main factors affecting it are the climatic conditions, the type and density of animal and plant populations, agricultural practices, and animal husbandry. The nitrogen cycle has undergone profound modifications through the agricultural and industrial activities of man (Bolin & Arrhenius, 1977; Committee on Nitrate Accumulation, 1972; Commoner, 1970; FAO/IAEA Panel of Experts, 1974).

Atmospheric nitrogen is in the form of dinitrogen (N_2); the great strength of the $N = N$ bond is mainly responsible for its chemical inertness. A part of atmospheric nitrogen is transformed by microbial action and incorporated into living organisms. This process is called nitrogen "fixation" and is estimated to amount globally to 150 million tonnes of fixed nitrogen per year. In industrial nitrogen fixation, atmospheric nitrogen is combined with hydrogen at high temperatures and pressures in the presence of suitable metal catalysts (Haber-Bosch process) to produce ammonia. Industrial nitrogen fixation accounts for about one quarter of the total world production of fixed nitrogen (Bolin & Arrhenius, 1977). Various atmospheric processes which have been discussed elsewhere (WHO, 1977) are minor sources of fixed nitrogen.

Biological nitrogen fixation, i.e. its reduction to ammonia, can be accomplished by only a limited number of organisms. Symbiotic nitrogen fixation takes place in the root nodules of legumes such as soya

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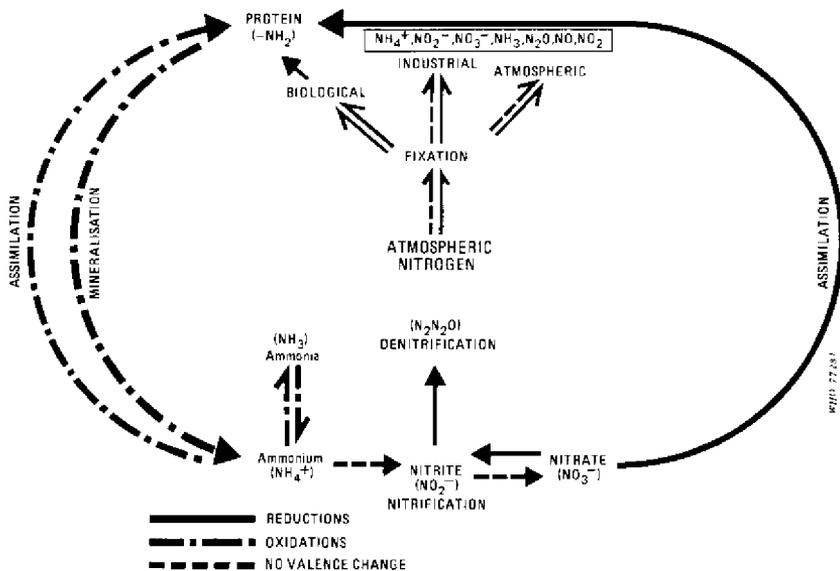
Fig. 1 The Nitrogen Cycle

From: Workshop on Global Ecology (1971)

bean, clover, and alfalfa, which contain bacteria of the *Rhizobium* species. There are also symbiotic processes with plants other than legumes involving, for example, some cyanobacteria. A number of free living bacteria and algae can also fix nitrogen (Burns & Hardy, 1975; Quispel, 1974). The fixation is catalysed by a complex enzyme nitrogenase (1.7.99.2). Ammonia produced by biological fixation is then converted to nitrite and nitrate by the process of nitrification (see section 2.1.1). Plants can assimilate only a part of the nitrates present in soils; some leaches into ground water and rivers and may reach estuaries and oceans, the rest is subjected to denitrification, another natural biochemical process that degrades nitrates to nitrogen or nitrous oxide (dinitrogen oxide) which are released into the atmosphere. Denitrification takes place in the soil and also at the interface between water and sediment in oceans, rivers, lagoons, and lakes. Nitrates from natural fixation and artificial fertilizers are ultimately used for the synthesis of biological molecules, particularly proteins. Plants and animal waste and dead tissues return fixed nitrogen to the soils, where part of it is recycled and part returned to the atmosphere, thus completing the nitrogen cycle. According to Delwiche (1970), nitrogen fixation on a world basis may exceed denitrification by about 10%. The increased use of industrial fertilizers has resulted in some areas in increased

concentrations of nitrates in bodies of water, resulting, in some cases, in eutrophication.

4.2   Transformation in Foods

4.2.1   Reduction of nitrates to nitrites

Because of the ability of spinach to accumulate large quantities of nitrates and reported cases of intoxication associated with the consumption of this vegetable, several studies have been undertaken on the conversion of nitrates to nitrites in spinach.

Data presented by Phillips (1968a) indicated that initial nitrite-N contents of fresh, frozen, canned, and baby-food spinach were generally less than 1 mg/kg fresh weight. However, several authors have reported a rapid fall in nitrate levels and increase in nitrite levels in fresh spinach during the first 4 days of storage at room temperature (Achtzehn & Hawat, 1970; Phillips, 1968a; Schuphan, 1965). Higher nitrite levels occurred in spinach from fertilized ground (Brown & Smith, 1967) and these could reach exceptionally high values (3600 mg/kg dry weight) with excessive fertilization (Schuphan, 1965).

Under refrigeration, the nitrite-N contents of fresh spinach increased very gradually throughout a storage period of 28 days (Phillips, 1968a). Significant increases in nitrite-N levels did not occur during the storage of frozen, canned, or baby-food spinach, but increased concentrations were found in frozen spinach, that had been left to thaw at room temperature for an excessively long period (39 h) (Phillips, 1968a).

There was also a slight rise in nitrite levels when partially consumed jars of commercial baby foods containing nitrates were stored for 7 days at room temperature instead of under refrigeration (Phillips, 1969). Selenka (1970) noted that nitrite formation in baby foods was rapid in the presence of *Escherichia coli* and *Pseudomonas fluorescens*, less rapid with *Bacillus subtilis*, and very slow with *Staphylococcus albus*.

When foods consisted of a solid immersed in a liquid (e.g. canned foods or frozen foods after thawing) nitrates were partially transferred to the liquid portion or into the water in which the food was cooked (Bodiphala & Ormrod, 1971). When large volumes of water and long cooking times were employed (Kilgore et al., 1963), and when canned vegetables were blanched in hot water instead of steam (Johnson, 1966), significant amounts of nitrates were leached out of the foods. Nitrate reductase (NADPH) (1.6.6.3) activity was rapidly destroyed during cooking, thereby greatly diminishing further conversion of nitrates to nitrites (Bodiphala & Ormrod, 1971). It is also well known that the

sterilization treatments necessary for canning destroy microorganisms that could convert nitrates to nitrites.

Conversion of nitrates to nitrites occurred more slowly in vacuum-packed bacon than in unpackaged bacon, presumably due to the low reducing ability of anaerobes (Cavett, 1962). Spencer (1967) found that the nitrite content of vacuum-packed bacon decreased slowly on storage. It has also been reported by Sebranek et al. (1974) that nitrite levels in meat, determined 2 days after processing, were less than half those originally added to frozen samples and samples processed at 71°C, and that they decreased further during storage. Frying, grilling, or boiling bacon or ham reduced the nitrites content by 20–90% (Food Standards Committee, 1959).

When direct gas firing of spray dryers was employed, the nitrate-N contents of dried milk products increased by 1–3 mg/kg compared with those obtained using indirectly heated sprayers but nitrite-N levels were unaffected (Manning et al., 1968). Air drying of potato and corn starch led to the formation of only trace amounts of nitrite (Gerritsen & De Willingen, 1969).

Nitrates may be reduced to nitrites when cooking is carried out in aluminium utensils (Osteryoung, unpublished data)[a]. This observation appears to be significant since some countries use aluminium utensils for boiling milk and water, a practice which could lead to the formation of sizeable quantities of nitrites. This effect of aluminium should be investigated further.

4.2.2 Formation and degradation of N-nitroso compounds

The conditions under which various amines, amino acids, and proteins in food could react with nitrite to form nitrosamines were studied by Ender & Čeh (1971) and by Sen et al. (1970) who showed that when cod, herring, hake, halibut, mackerel, or salmon were treated with sodium nitrite at 200 mg/kg and cooked at 110°C for 60–70 min there were only trace amounts (2.5 25 µg/kg) of DMN in the cooked product. The highest levels were found in mackerel and hake, both of which contain large amounts of DMA and TMA. Samples without added nitrite did not contain any detectable nitrosamines.

The formation of DMN was studied in aqueous model systems containing methyl amines and sodium nitrite under conditions which were more severe than those employed in the commercial processing of

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[a]Nitrates as human and animal health hazards, Paper presented at the Second Conference on Environmental Chemicals, Colorado State University, 1973.

nitrite-treated smoked chub (a fresh water fish containing small amounts of TMA, TMAO, and DMA). The results of the studies showed that not more than 10 µg of DMN per kg of the final product would be formed during the smoking process (Malins et al., 1970).

Recently, Sen et al. (1973b) suggested that a major source of nitrosamines in cured meat might arise from an interaction between the nitrites and spices, such as black pepper and paprika, that are present in curing mixtures. Nitrosopyrrolidine, nitrosopiperidine, and DMN were found in a curing mixture used by a meat manufacturer in Canada. The same authors (Sen et al. 1974a) have also studied the effect of sodium nitrite concentration on the formation of nitrosopyrrolidine and DMN in fried bacon. Bacon samples prepared with sodium nitrite at 0, 50, 100, 150, or 200 mg/kg were analyzed for nitrosopyrrolidine and DMN. No nitrosamine was detected in samples prepared without nitrite but all treated samples contained 2–20 µg/kg of nitrosamines. The level of nitrosopyrrolidine was related to the initial concentration of nitrite in the bacon. It has also been shown that nitrosamine formation in bacon increases with increasing temperature and time of frying and that whereas baking, broiling, or frying produce variable amounts of nitrosamines none is produced with cooking in a microwave oven (Pensabene et al., 1974).

After deliberate nitrosation of eggs and meat with unusually large amounts (1%) of sodium nitrite, some N-nitroso compounds appeared to have been formed but the chemical nature of the compounds detected was not clear (Walters, 1971).

No systematic studies on the formation of N-nitroso compounds in cheese have been performed, although some types of cheese are known to be processed with nitrate and nitrite.

There are few data on the fate of N-nitroso compounds during the cooking, processing, or storage of food but some studies have demonstrated that the volatile nitrosamine DMN and nitrosopyrrolidine may be lost during the frying of bacon (Sen et al., 1973a).

4.3 Formation of N-nitroso Compounds from Drugs and Pesticides

Reaction with nitrite to form nitrosamines is not restricted to food components. Lijinsky & Greenblatt (1972) and Lijinsky (1974) reported that some antibiotics and other drugs that are widely used can react with nitrites to form nitrosamines in alarmingly high quantities. The drugs examined included oxytetracycline, aminophenazone (aminopyrine), disulfiram, N,N-diethyl-3-pyridinecarboxamide (nikethamide), tolazamide, and (E,E)-1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]

piperidine (piperine). Optimum conditions of temperature, pH, and concentration for these reactions have been reported by Lijinsky et al. (1972a, 1972b, 1972c) who more recently (Lijinsky, 1974) studied the reactions of aminopyrine and other commonly used drugs with nitrous acid at rather low concentrations to assess the magnitude of the hazard to man from such interactions. The topic has been reviewed by Mirvish (1975) who has listed 41 drugs and pesticides that have been nitrosated. Pesticides listed include atrazine, simazine, ziram, and thiram.

4.4   Formation of N-nitroso Compounds in Animal Organisms

4.4.1   Formation of N-nitroso compounds in simulated gastric juice

Formation of DEN was demonstrated when DEA and nitrite were incubated in the gastric juice of the rat, rabbit, cat, dog, and man. More DEN was formed in human and rabbit gastric juices (pH 1–2 in both cases) than in rat gastric juice (pH 4–5). (Sen et al., 1969a, 1969b).

The formation of nitrosamines by the interaction of some drugs with nitrite in the presence of human gastric juice have been studied by Scheunig & Ziebarth (1976). At 37°C and a pH = 2, for 1 h, aminopyrine, sodium [(2,3 dihydro-1,5-dimethyl-3-oxo-2-phenyl-1H-pyrazol-4-yl)methylamino] methanesulfonate (analgin), and piperazine gave nitrosamine yields (calculated on the basis of nitrite used) of 69%, 11%, and 74.8% respectively.

In vitro studies have been carried out (Wells et al., 1974) in which several foods (pork, egg, bread, milk, and cheese) were incubated under simulated gastric conditions with concentrations of nitrite similar to those used as food preservatives. The effect of the thiocyanate ion as a catalyst for nitrosation was also studied since it is secreted in saliva. Of the foods studied, only cheese produced detectable amounts of volatile nitrosamines. The identity of the nitrosamines was not indicated.

4.4.2   Formation of N-nitroso compounds *in vivo*

When DEA and nitrite were fed to cats and rabbits, considerable amounts of DEN were detected in the stomach of the experimental animals (Sen et al, 1969a, 1969b). Similar results were reported by Sander & Sief (1969). Epstein (1972) reported the formation of nitrosopiperidine in the gastrointestinal tract of rats treated with nitrite and piperidine hydrochloride. When the nitrite concentration was constant, nitrosopiperidine formation in the small intestine increased with increasing concentrations of piperidine. Nitrosopiperidine was also found in the stomach. Recently, Sander et al. (1974a) demonstrated the

formation of N-N'-dinitrosopiperazine, DMN, and N-nitroso-N-methylbenzylamine in the stomach contents of rats given the parent amines combined with nitrite. Considerable individual variation in the degree of synthesis of N-N'-dinitrosopiperazine was noted in the animals. In another recent report, N-nitrosopyrrolidine was formed very rapidly in the stomach of dogs from sodium nitrite and pyrrolidine (within 2–6 min) but after 30 min nearly all of it had disappeared, presumably due to its rapid absorption (Mysliwy et al., 1974).

Indirect evidence of *in vivo* formation of N-nitroso compounds has also been provided by some toxicity studies. Thus, hepatic lesions, formed following administration of nitrite and some amines, were similar to those produced by DMN or N-nitroso-N-methylbenzylamine (Asahina et al., 1971). Similar effects were noted where nitrite was administered up to 3 h after DMA, but the effect was markedly reduced if the nitrite was given prior to the amine.

4.5 Formation of N-nitroso Compounds by Microorganisms

Studies conducted by Hawksworth & Hill (1971a), Klubes & Jondorf (1971), and Sander & Sief (1969) suggested that nitrosamines could be synthesized from secondary amines and nitrates or nitrites by *Escherichia coli* and some species of streptococci. Fong & Chan (1973b) demonstrated that homogenized Chinese salt fish inoculated with *Staphylococcus aureus* (a nitrate-reducing bacterium) produced considerable amounts of DMN.

Formation of nitrosamines in the presence of bacteria is unlikely to occur in the large intestine, but the infected bladder and achlorhydric stomach are likely sites (Hawksworth et al., 1974).

The ultimate mechanism of bacterial production of nitrosamines remains to be ascertained. According to Hawksworth et al. (1974), certain bacteria do reduce nitrate to nitrite but the formation of the nitrosamine may be nonenzymatic and involve some heat-resistant metabolite.

4.6 The Effects of Other Chemicals on the Formation of N-nitroso Compounds

Fiddler et al. (1973) and Greenberg (1974) showed that high levels of ascorbic acid reduced nitrosamine formation in frankfurter sausages and in fried bacon. On the other hand, Nagata & Mirna (1974) reported an

increase in nitrosamine formation in meat products in the presence of ascorbic acid. Other studies conducted on the inhibition of nitrosamine formation by various compounds include a report by Sen & Donaldson (1974) in which nitrosamine formation in human saliva was inhibited by ascorbic acid. Ziebarth & Scheunig (1976) tested a number of substances and beverages for the inhibition of the nitrosation of several drugs under simulated gastric conditions. Of all the substances investigated, ascorbic acid was regarded as the best inhibitor because of its pronounced activity at pH values occurring in the stomach and because it was not toxic in the amounts used.

5. ENVIRONMENTAL LEVELS AND EXPOSURES

5.1 Nitrates and Nitrites

5.1.1 Ambient air

Nitrate aerosols are the final stage in the atmospheric oxidation of gaseous oxides of nitrogen, and substantial amounts of particulate nitrates may be formed in urban areas affected by photochemical pollution (Pitts & Lloyd, 1973). The concentration of nitrates in air may range from about 1 to 40 $\mu g/m^3$, depending on the sampling and averaging periods. For example, the estimated annual mean values (1968–1972) in Chattanooga, TN, USA, were between 1 and 6 $\mu g/m^3$ (French et al., unpublished)[a]. The daily mean concentrations of airborne nitrates in the central part of Tokyo ranged, in 1973, from 0.9 to 41.8 $\mu g/m^3$ with an annual mean of 8.2 $\mu g/m^3$. On the other hand, in a small city with few industries (Matsue City) the daily means were in the range of 1.1 – 9.2 $\mu g/m^3$, with an annual mean of 2.6 $\mu g/m^3$ (Japan Environmental Sanitation Center, 1974).

5.1.2 Water

The concentrations of nitrates and nitrites in surface and ground waters vary within wide limits, depending on geochemical conditions, human and animal waste management practices, the extent to which nitrogen-containing agriculture fertilizers are used locally, and on industrial discharges of nitrogen compounds (section 3.2.1.).

In general, surface waters do not usually contain nitrate in concen-

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[a] French, J. G., Hasselblad, V., & Johnson, R. Aggravation of asthma by air pollutants. 1971–72 Southeastern CHESS studies.

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trations higher than 10 mg/litre, and nitrite concentrations rarely exceed 1 mg/litre. However, a steady upward trend of nitrate levels has been reported in recent years in some countries, both in surface and ground waters. Thus, for example, in the River Thames, England, nitrate concentrations increased from an average of 4 mg/litre in 1968 to an average of 9 mg/litre for the last quarter of 1973 (Water Research Centre, 1974). Similar increases have been observed in several other English rivers (Casey, 1975; Owens, 1970; Tomlinson, 1970). The nitrate concentrations are increasing in some rivers that drain the great agricultural section of the centre of the USA, and in selected small rivers the 45 mg/litre limit is sometimes exceeded (Viets & Aldrich, 1973). A small increase in the nitrate concentration of the Tamagawa River, Tokyo, Japan has also been reported. From 1951–1965, the nitrate ion concentration rose from 7.9 mg/litre to 9.1 mg/litre. During the same period, the nitrite concentration increased from 0.049 mg/litre to 0.53 mg/litre, i.e by a factor of about 10 (Goto, 1973).

Studies of 991 settlements in Bulgaria indicated that only 64 towns and villages had drinking water levels of nitrates between 30 mg/litre (Bulgarian standard) and 50 mg/litre. In 20 settlements, situated in areas with intensive agriculture and stock breeding, the nitrate concentrations exceeded 50 mg/litre. The report[a] points out that such problems did not exist some 10 years ago when smaller quantities of nitrogen fertilizers were used in agriculture.

Much higher concentrations of nitrates are sometimes found in ground water, particularly in water derived from dug wells. A survey of over 2000 rural wells in Saskatchewan, Canada, revealed that 18.8% contained nitrate concentrations of more than 50 mg/litre and 5.3% had nitrate levels exceeding 300 mg/litre (Robertson & Draycott, 1948). Hedlin (1971) also reported levels above 45 mg/litre in some wells in a rural area of Manitoba, Canada. In many farm wells in central USA, nitrate concentrations may range from 45–450 mg/litre. This problem is neither new nor local, since such conditions have been recorded from 1895 to 1970 in Illinois, in 1939 in Iowa, and in 1970 in Minnesota (Viets & Aldrich, 1973). The mean nitrate concentration in ground water consumed by children affected by methaemoglobinaemia in Czechoslovakia ranged from 18–257 mg/litre (Schmidt & Knotek, 1970). According to Gruenar & Shuval (1970), about 180 wells for community water supplies in the densely populated central and southern coastal plain in Israel had nitrate concentrations exceeding 45 mg/litre. In England,

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[a]Contribution to the WHO environmental health criteria document on nitrates, nitrites and nitrosamines, Sofia, 1974.

nitrate concentrations in some ground waters have been reported to range from 12 mg/litre (Foster & Crease, 1974) to over 22 mg/litre (Reeves et al., 1974). Nitrate concentrations exceeding 45 mg/litre have not been reported in centralized water supplies in the USSR[a]. However, high concentrations have been found from time to time in dug wells, for example, 310 400 mg/litre in Leningrad Oblast (Motylev, 1969), 110–200 mg/litre in the Tatar SSR (Petukhov et al., 1972) and up to 430 mg/litre in the Moldvian SSR (Diskalenko, 1969).

5.1.3 Selected foods

According to the data compiled by the National Institute of Environmental Health Sciences (NIEHS, 1970), the levels of nitrates in vegetables vary considerably. The highest levels were found in beets, egg plant, kale, and spinach and the lowest in tomatoes and peas; similar findings were obtained in the German Democratic Republic by Achtzehn & Hawat (1969). It is of interest to note that the nitrate levels in vegetables reported by Jackson in 1967 were similar to those reported by Richardson in 1907, when manure was used instead of chemical fertilizers.

Nitrate contents vary not only between vegetable species but also widely within a given species. This variation within a species may be accounted for by such factors as temperature, sunlight, soil moisture, and the level of available nitrogen in the soil (US Department of Agriculture, 1965). A relationship between nitrate accumulation in spinach and levels of fertilizer applied to the soil has been demonstrated by a number of authors (Brown & Smith, 1967; Phillips, 1971). Furthermore, Schuphan (1965) reported exceptionally high levels of nitrites (3600 mg/kg dry weight) in excessively fertilized fresh spinach stored at room temperature.

A survey of the nitrate contents of fruits in the German Democratic Republic, revealed that they were high in bananas and strawberries but could not be detected in the other fruits examined (Achtzehn & Hawat, 1969).

Cow's milk contained nitrate levels of 0–0.5 mg/litre (Simon et al., 1964).

The levels of nitrates and nitrites in baby foods are of special concern since infants are considerably more sensitive to the toxic effects of nitrates than adults. Kamm et al. (1965) studied 194 prepared infant

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[a] Contribution to the WHO environmental health criteria document on nitrates, nitrites and nitrosamines, Moscow, 1974.

foods and found that, on average, fruits, dairy products, puddings, egg products, meats, dry and concentrated food supplements, and precooked cereal products contained nitrate levels of less than 90 mg/kg. Vegetables, however, had a wide range of nitrate contents varying from 0.9 to 2165 mg/kg, but nitrite levels never exceeded 7 mg/kg. In studies on a number of canned foods, baby foods, frozen foods, and vegetables, several varieties of fruit, spinach, and beets generally had the highest nitrate contents (Bodiphala & Ormrod, 1971). Additional information may be found in articles by NIEHS (1970) and Ashton (1970).

In a survey of various cured meats (Table 1, Ashton, 1970), the highest nitrate content of 370–511 mg/kg was found in ham. In analysing 197 samples of cured meat products, Panalaks et al. (1972) found that the levels of nitrates and nitrites ranged from 0–3467 mg/kg and 0–252 mg/kg, respectively.

Dubrow & Kakisch (1960) analysed 338 samples of cheese and reported that all were free of nitrites (less than 1 mg/kg). However, 40% of the samples contained nitrate levels of more than 1 mg/kg. Rammell & Joerin (1972) also found low nitrite levels in cheese.

Table 1. Nitrate and nitrite contents of cured meats

Meat type	Nitrate (mg/kg)	Nitrite (mg/kg)
silverside	133–303	9 – 26
ham	370–511	7 –150
luncheon meat	59–214	3.1– 47
chopped ham & pork	53–101	22 – 62
corned beef	118–135	18 ·208
frankfurter sausage	119–141	8.5– 10.3

From: Ashton (1970)

5.1.4 Estimate of general population exposure

One of the important sources of exposure to nitrates for man is water. The level of nitrates in water may vary from practically nil to over 200 mg/litre. In water from municipal supplies, however, it is likely to be under 10 mg/litre. Thus, assuming an intake of 2 litres of water per day, the daily intake of nitrates from this source would normally be less than 20 mg, but with extremes of 0 and over 400 mg.

The other main sources of nitrates and nitrites are certain vegetables and meat products. The intake from these sources is even more variable because of marked differences not only in levels in these foods but also in dietary patterns. However, Ashton (1970) estimated the weekly intake of

nitrates for a member of the general population in the USA to be about 400 mg including 210 mg from vegetables, 110 mg from meat products, and 85 mg from water (7 litres per week). The estimate of Hill et al. (1973) for a member of the general population in England included 225 mg from vegetables, 110 mg from meat, and 105 mg from water in "control towns" and 645 mg from water in Worksop, England. However these figures cannot be applied generally because of variations in feeding habits and nitrate levels in environmental media. Since the intake of nitrites is even more variable, no estimates have been reported.

5.2 *N*-nitroso Compounds

5.2.1 Ambient air

The occurrence of *N*-nitroso compounds in urban air was reported first by Bretschneider & Matz (1973) and confirmed by Fine et al. (1976b) who found DMN at concentrations of about 1.2–3.5 $\mu g/m^3$ (0.33–0.96 ppb) in an industrial district in Baltimore, MD, USA, and about 0.06–0.17 $\mu g/m^3$ (0.014 ppb–0.051 ppb) in Belle, WV, USA. *N*-nitroso compounds may be present in air, either due to their formation in the air from secondary amines and oxides of nitrogen (Neurath, 1972) or due to industrial omissions as in the instances referred to.

5.2.2 Water

There are few reports on the occurrence of *N*-nitroso compounds in water. Fine et al. (1976b) analysed samples from the Mississippi river (New Orleans, LA) and from 3 water treatment plants in Louisiana. Using the new *N*-nitroso compound-specific thermal energy analyser (TEA) interfaced to both a gas chromatograph (GC) and a high performance liquid chromatograph (LC), several peaks were tentatively identified as belonging to *N*-nitroso derivatives of some pesticides. The estimated concentrations were of the order of 0.1 $\mu g/kg$.

5.2.3 Selected foods

A summary of the reported occurrences of nitrosamines in meat and fish products, adapted from Sen (1974) and updated, is presented in Table 2. Only the results that were confirmed by mass spectroscopy are quoted in this table. It was noted that the majority of some 50 publications dealt with the determination of nitrosamines, particularly DMN, in processed pork meat. The methods employed for analysis mainly involved gas chromatography. A few results were confirmed by

Table 2. Levels of nitrosamines in various meat and fish products[a]

Meat	Country or area	N-nitroso compounds found[b]	Levels ([c])	Reference
dry sausage	Canada	DMN	10-20 µg/kg	Sen (1972)
uncooked salami sausage	Canada	DMN	20-80 µg/kg	Sen (1972)
salami sausage	Netherlands	DMN	0.3 µg/kg([c])	Stephany et al. (1976)
		DMN	0.1([c])	Stephany et al. (1976)
		NDBA	1.1([c])	Stephany et al. (1976)
	Netherlands	NPY	0.4([c])	Stephany et al. (1976)
		NPIP	0.3([c])	Stephany et al. (1976)
bacon	Canada	NPY	4.25 µg/kg	Sen et al. (1973a)
	Canada	NPY	25-40 µg/kg	Sen et al. (1974a)
bacon	Netherlands	DMN	0.8 µg/kg([c])	Stephany et al. (1976)
		DEN	0.2([c])	
		NDBA	0.6([c])	
	Netherlands	NPY	0.4([c])	Stephany et al. (1976)
		NPIP	0.6([d])	
	Netherlands	NPY	7-35 µg/kg	Stephany et al. (1976)
bacon	USA	NPY	2, 28, 13	Pensabene et al. (1974) Fiddler et al. 1974
uncooked bacon	Canada	DMN	30 µg/kg	Sen et al. (1973a)
fried bacon	Netherlands	DMN	2.4 µg/kg	Groenen et al. (1976)
		DEN	4.43 µg/kg	
	Netherlands	DMN	1.1 µg/kg([c])	Stephany et al. (1976)
		DEN	0.2([c])	
		NDBA	0.7([c])	
	Netherlands	NPY	16.4([c])	Stephany et al. (1976)
		NPIP	3.9([c])	
	Netherlands	NPY	1-40 µg/kg	Stephany et al. (1976)
	UK	NPY		Crosby et al. (1972)
	USA	NPY	20-207 µg/kg	Fazio et al. (1973)
smoked meat	Netherlands	DEN	7.91 µg/kg	Groenen et al. (1976)
	Netherlands	DMN	3	Groenen et al. (1976)
smoked horse and beef meat	Netherlands	DMN	7.3 µg/kg([d])	Stephany et al. (1976)
		DEN	0.6([d])	Stephany et al. (1976)
		NDBA	0.4([d])	Stephany et al. (1976)
	Netherlands	NPY	0.1([d])	Stephany et al. (1976)
	Netherlands	NPIP	0.1([d])	Stephany et al. (1976)

Table 2.—Continued

Description	Country	Compound	Value	Reference
ham with layer of pepper grains on the outside. Only fat portion.	Germany (Federal Republic of)	DMN	3 µg/kg	Eisenbrand et al. (1975)
ham with layer of pepper grains on the outside. Whole product homogenized	Germany (Federal Republic of)	NPIP	6 µg/kg	Eisenbrand et al. (1975)
	Germany (Federal Republic of)	NPY	6	
ham, fried	Germany (Federal Republic of)	DMN / NPIP / NPY	1 µg/kg / 8 / 19	Eisenbrand et al. (1975)
ham with layer of pepper grains on the outside. Whole product homogenized.	Germany (Federal Republic of)	NPIP	4 µg/kg	Eisenbrand et al. (1975)
	Germany (Federal Republic of)	NPY	9	
German type of bacon, raw	Germany (Federal Republic of)	DMN	1 µg/kg	Eisenbrand et al. (1975)
German type of bacon, fried	Germany (Federal Republic of)	DMN / NPIP / NPY	1 µg/kg / 5 / 19	Eisenbrand et al. (1975) / Eisenbrand et al. (1975) / Eisenbrand et al. (1975)
smoked raw meat	Germany (Federal Republic of)	DMN	2 µg/kg	Eisenbrand et al. (1975)
smoked ham	Germany (Federal Republic of)	DMN	8 µg/kg	Eisenbrand et al. (1975)
smoked ham	USA	DMN	5 µg/kg	Fazio et al. (1971b)
smoked ham	USA	DMN	5 µg/kg	Fiddler et al. (1974)
cooked and smoked ham	Netherlands	DMN / DEN / NDBA	0.4 µg/kg(f) / 0.6( ) / 0.4( )	Stephany et al. (1976) / Stephany et al. (1976) / Stephany et al. (1976)
ham	Netherlands	NPY / NPIP	0.3( ) / 0.4( )	Stephany et al. (1976) / Stephany et al. (1976)
cooked ham	Netherlands	DMN	6 µg/kg	Groenen et al. (1976)
Bologna sausage	Canada	DEN	25 µg/kg	Panalaks et al. (1974)
	Canada	NPY	20, 100, 105	Panalaks et al. (1974)
frankfurter sausage	USA	NPIP	50, 50, 60 µg/kg	Wasserman et al. (1972)

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Table 2. Continued

spiced meat	Canada	DMN	5–48 µg/kg	Sen et al. (1976)
products	Canada	DEN	6–16	
	Canada	NPIP	14–50	Sen et al. (1976)
		NPY	7–33	Sen et al. (1976)
fish meal	Canada	DMN	0.35–0.5 mg/kg	Sen et al. (1972)
smoked, nitrate/	USA	DMN	4–26 µg/kg	Fazio et al. (1971a)
or nitrite treated				
sable, salmon shad				
fresh, smoked or	UK	DMN	1–9 µg/kg	Crosby et al. (1972)
salted fish				
salted white	Hong Kong	DMN	40–100 µg/kg	Fong & Chan (1973a, 1973b)
herring				
salted yellow	Hong Kong	DMN	10–60 µg/kg	Fong & Chan (1973a, 1973b)
croakers				
crude salt salted	Hong Kong	DMN	400 µg/kg	Fong & Chan (1973a, 1973b)
white herring				
crude salt salted	Hong Kong	DMN	200 µg/kg	Fong & Chan (1973a, 1973b)
yellow croakers				
prime salt salted	Hong Kong	DMN	10 µg/kg	Fong & Chan (1973a, 1973b)
white herring				
prime salt salted	Hong Kong	DMN	5 µg/kg	Fong & Chan (1973a, 1973b)
yellow croakers				
salted anchovies	Hong Kong	DMN	20 µg/kg	Fong & Chan (1973a, 1973b)
whole herring meal	Hong Kong	DMN	300 µg/kg	Fong & Chan (1973a, 1973b)

[a] Adapted from Sen (1974)
[b] DMN — N-methyl-N-nitrosomethanamine
DEN — N-ethyl-N nitrosoethanamine
NDBA — Nitroso-N-butylamine
NPY — Nitrosopyrrolidine
NPIP — Nitrosopiperidine

[c] All values confirmed by mass spectroscopy
[d] mean of 4 samples
[e] mean of 5 samples
[f] mean of 6 samples
[g] mean of 10 samples

mass spectroscopic techniques. However, mass spectroscopy confirmation is currently being employed more frequently than in the past.

Several authors who detected nitrosamines in foods by screening methods but did not confirm these results by mass spectrometry include Ender et al. (1964, 1967), Ender & Čeh (1967), Fong & Walsh (1971), Freimuth & Gläser (1970), Hedler & Marquardt (1968), Kröller (1967), Lembke & Moebus (1970), Möhler & Mayrhofer (1968, 1969), and Sakshaug et al. (1965). Nevertheless, results by screening methods should not be entirely ignored.

Considerable variations have been found in the levels of volatile N-nitroso compounds in fried and grilled bacon. Attempts to correlate these levels with levels of nitrates and nitrites did not reveal any definite pattern. Telling et al. (1974) studied the effect of various cooking temperatures on the levels of N-nitroso compounds in grilled bacon. The results indicated that the levels of DMN remained fairly constant as the cooking temperature was raised but those of nitrosopyrrolidine increased.

Lipid soluble nitrosamines have an affinity for the fatty portions of food (Sen et al. 1973a).

5.2.4   Tobacco and tobacco smoke

Since precursors for the formation of nitrosamines occur in tobacco, Druckrey & Preussman (1962) thought it likely that tobacco or tobacco smoke might contain trace amounts of nitrosamines. Initially, studies on the nitrosamine content of tobacco products were hampered due to interference from other compounds. Later, evidence suggesting the presence of DMN, nitrosopyrrolidine, methylbutylnitrosamine, and nitrosopiperidine in tobacco smoke was obtained (Kröller, 1967; Neurath et al., 1964; Neurath, 1972, Serfontein & Hurter, 1966). Although anabasine and nornicotine are constituents of tobacco smoke, the corresponding nitroso derivatives were not detected by Neurath (1972). Recently, however, Hoffman et al. (1974) reported the presence of N-nitrosonornicotine at levels of up to 88 mg/kg in unburned tobacco.

5.2.5   Estimate of general population exposure

The N-nitroso compounds that have been identified and determined in meat and fish are listed in Table 2; consumption of these foods constitutes a definite exposure of the general population to these chemicals. However, at present, no estimate can be made of the human exposure from these and other sources because insufficient samples have

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been analysed and because the relevant food consumption surveys have not been made.

5.2.6 Occupational exposure to *N*-nitroso compounds

The potential occupational hazards associated with the use or manufacture of *N*-nitroso compounds in industry have been pointed out by Magee & Barnes (1967).

Only a few quantitative data are available, however, on the concentration of *N*-nitroso compounds in the work environment. In a study by Bretschneider & Matz, (1976) DMN levels in the air in a factory manufacturing DMA were roughly estimated to range from 0.001 to 0.43 $\mu g/m^3$.

6. METABOLISM OF NITRATES, NITRITES, AND *N*-NITROSO COMPOUNDS

6.1 Gastrointestinal Absorption

6.1.1 Nitrates and nitrites

A part of ingested nitrates is readily absorbed and a part may be metabolized by the microflora in the gastrointestinal tract (Ridder & Oehme, 1974). Nitrites (NO_2^-), oxides of nitrogen (N_2O_5, NO_2, NO), hydroxylamine ($NH_2 OH$), and ammonia (NH_3) can be formed depending upon the organisms present, the pH, and the available nutrients (trace elements and carbohydrates), and may be absorbed.

Friedman et al. (1972) gave mice a single oral dose of 150 μg of sodium nitrite. Measurement of the rate of disappearance showed that the compound was rapidly absorbed and that food in the stomach had little effect on absorption. Results using animals with a ligated gastro-duodenal junction suggested that the major absorption site was the gastric mucosa. Studies by Mirvish et al. (1974) on rats fed a diet containing nitrite supported the previous observations on mice. Experiments with food containing phenol red showed that a decrease in nitrite levels in the stomach contents, especially in the glandular part, that occurred within 5 h of feeding, was significantly greater than that due to direct faecal elimination. This was attributed to decomposition and other acid-catalyzed reactions of nitrite and to direct absorption from the stomach.

6.1.2 *N*-nitroso compounds

Although a considerable degree of absorption of nitrosamines can be inferred from the nature of their toxic effects following oral administration, few reports could be found giving quantitative information on absorption. Alarif & Epstein (1974) gave ^{3}H-labelled nitrosomethylurea and nitrosomethylurethane by gavage to groups of pregnant guineapigs at doses of 2 mg/kg, and 5 mg/kg body weight, respectively. The animals were killed 1 h after dosing. When the uptake by maternal and fetal tissues was measured by liquid scintillation counting and DNA determination, maternal levels were generally higher than fetal levels. In studies by Juszkiewicz & Kowalski (1974) DMN, DEN, and nitropropylamine, administered orally to goats at 20–30 mg/kg, appeared in the blood within $\frac{1}{2}$ h, and later in the milk. The concentration in the milk, 2 h after administration of DEN at 30 mg/kg, was 14 mg/litre; after 24 h only traces could be found. Phillips et al. (1975a) examined the disappearance of DMN from the stomach and small intestine of rats as an index of absorption, and found that, while very little was absorbed from the stomach, DMN was rapidly absorbed from the small intestine.

6.2 Biotransformation and Elimination

6.2.1 Nitrates and nitrites

In a study on 4 rats, 42–90% of nitrates, administered by stomach tube, was excreted in the urine within 8 h of administration. Nitrites were not detected in the urine either before or after administration (Hawksworth & Hill, 1971b). The same authors carried out a study on 122 samples of human urine, and found that urinary nitrate concentration was related to the amount of nitrate ingested.

The excretion of nitrates and nitrites in the saliva was studied by Spiegelhalder et al. (1976) in 11 volunteers given various vegetable juices with nitrate concentrations ranging from 30 to 550 mg/litre. The levels of nitrates and nitrites excreted were proportional to the amounts of nitrates ingested. After ingesting 100 mg of nitrates, nitrite concentrations in the saliva increased, on average, by 20 mg/litre.

6.2.2 *N*-nitroso compounds

Magee & Barnes (1967) have reviewed available information on the metabolism and elimination of *N*-nitroso compounds. Magee (1956) measured recovery of DMN from the whole body of the mouse and noted that 97% of the total dose (0.05 mg/kg) could be recovered

immediately after oral administration and that the amounts recovered decreased with time until at 4 h no DMN was recovered. Similar results were obtained with the rat given DMN orally at 50 mg/kg; the concentration fell rapidly with increasing time after injection so that only 30% of the dose was recovered at 8 h and none at 24 h.

Metabolic transformation of DMN was demonstrated by Dutton & Heath (1956) using ^{14}C-labelled DMN. In both the mouse and the rat, the main radioactive product was expired carbon dioxide. In the mouse, 65% of the injected ^{14}C was recovered as expired carbon dioxide, 6 h after a subcutaneous injection of DMN at 50 mg per kg body weight. In the rat, about 40% of the radioactive ^{14}C was recovered as expired carbon dioxide, 8 h after the injection. At the end of the experiment, the remainder of the ^{14}C was fairly evenly distributed in the tissues, apart from about 7% that was excreted in the urine.

Heath (1962) found that, while part of each of a number of nitrosamines studied was excreted unchanged in urine and in expired air, the greater part was decomposed. From the rates of expiration of labelled carbon dioxide it was shown that decomposition of dimethyl-, diethyl-, and N-butylmethylnitrosamine obeyed Michaelis-Menten kinetics. The rate of decomposition was dose-dependent.

When the metabolic transformation of dialkylnitrosamines, especially of the bladder carcinogen di-N-butylnitrosamine was investigated, major urinary metabolites with retained N-nitroso structure were identified (Blattmann & Preussman, 1973, 1974; Blattmann et al., 1974; Okada & Suzuki, 1972; Okada et al., 1975). Hydroxylation, particularly at the terminal CH_3 group, has been observed as well as chain shortening. Butyl (3-carboxypropyl) nitrosamine (BCPN), a major metabolite of butyl (4-hydroxy butyl)-nitrosamine (BBN) was an equally potent and selective bladder carcinogen (Okada & Suzuki, 1972). Ring-opening was observed during metabolism of nitrosomorpholine (Stewart et al., 1974).

7. EXPERIMENTAL STUDIES ON THE EFFECTS OF NITRATES, NITRITES, AND N-NITROSO COMPOUNDS

7.1 Nitrates and Nitrites

7.1.1 Acute and subacute toxicity studies

Acute nitrate poisoning was first recognized in cattle[a] by Mayo as early as 1895 (Wright & Davidson, 1964), while Comly (1945) was the

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[a] For further discussion of nitrate intoxication in livestock, that may involve significant economic loss, see Oehme (1975).

first to report nitrate poisoning from well water in infants in the USA. As a result of these and other reports, several studies have been conducted on the toxicity of nitrates in a wide variety of animal species. They are mainly centred on the formation of methaemoglobin that accompanies excessive exposure to nitrates and nitrites. The acute toxicity of nitrates and nitrites was recently reviewed by the Committee on Nitrate Accumulation (1972).

Although the outstanding feature of nitrate toxicity is the development of methaemoglobinaemia, nitrates may also cause vasodilation which aggravates the effects of the methaemoglobinaemia. The nitrite ion formed by reduction of nitrates, oxidizes the iron in the haemoglobin molecule from the ferrous to the ferric state. The resultant methaemoglobin is incapable of reversibly binding oxygen (Bosch et al., 1950). Clinical signs of nitrate toxicity, attributable to hypoxia appear when methaemoglobin values exceed about 20% (section 8.1). Oxidation of haemoglobin to methaemoglobin by the nitrite ion occurs at different rates for each animal species, but there is little difference between individuals of the same species (Smith & Beutler, 1966). Similarly, the reduction of methaemoglobin in erythrocytes mainly by the enzyme system, NADH--methaemoglobin reductase, is characteristically different for each animal species. The chemical induction of methaemoglobinaemia has recently been reviewed by Smith (1969, 1975). These physiological processes appear to be related, even though there is a large variation in the rate of formation of methaemoglobin and its subsequent reduction. This may help to explain the difference in species susceptibility and the variation in signs seen in nitrate poisoning.

In an effort to develop sensitive tests for the detection of the possible effects of subclinical methaemoglobinaemia, behavioural studies with mice were undertaken by Behroozi et al., 1971. Groups of 57 black, 6J, male mice were given nitrites in their drinking water at doses aimed at producing methaemoglobin levels varying from slightly above normal to 15%, which can be considered to be in the subclinical range. Sodium nitrite doses in water were 100, 1000, 1500, or 2000 mg/litre. The results showed a significant reduction of overall motor activity in the groups receiving the highest levels of nitrites. There was a significant inverse relationship between the methaemoglobin level and motor activity, with a coefficient of correlation of 0.65. An effort to counteract the methaemoglobinaemia was made by giving ascorbic acid to the group receiving the highest level of nitrites (2000 mg/litre). (See section 7.1.5). The effect was to reduce the methaemoglobin levels to almost normal but the motor activity level of the group so treated remained low and about equal to the equivalent group that had not received an antidote. These

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experiments seemed to indicate that the nitrites had some form of sedative effect on the treated mice, that was not necessarily associated with the development of methaemoglobinaemia.

Experiments on rabbits have shown that methaemoglobinaemia caused by nitrates in water also affects cardiac activity by increasing the number of cardiac contractions to an extent directly proportional to the increase in methaemoglobin levels. At the 10–15% methaemoglobin level, the electrocardiogram shows a shortening of the Q–T interval and a reduction in the T wave, which may even become negative (Garbuz, 1968, 1971).

7.1.2 Chronic toxicity and carcinogenicity studies

In a study conducted by Shuval & Gruener (1972), groups of 8 male rats (3 months old) were given tap water (control) or 100, 1000, 2000, or 3000 mg of sodium nitrite per litre of drinking water. After 24 months, there were no significant differences in growth and development, mortality, and total haemoglobin levels between the control and treated groups. However, the methaemoglobin levels in the groups receiving sodium nitrite at 1000, 2000, or 3000 mg/litre were raised significantly throughout the study and averaged 5%, 12%, and 22% of total haemoglobin respectively. The methaemoglobin levels in the group receiving 100 mg/litre were slightly above those of the control group for the first 60 days only. There were some changes in the liver and spleen of treated animals but the main pathological changes occurred in the heart and lungs. In the heart, small foci of cells and fibrosis were seen in some animals with pronounced degenerative foci in animals receiving the highest concentrations of nitrites. The coronary arteries were thin and dilated. The bronchi were frequently dilated with the walls infiltrated by lymphocytes and the mucosa and muscle were often atrophied. Emphysema was the rule. These changes, which were present in 1 or 2 control rats and in a small number of those receiving nitrite levels of 100 mg/litre, were found with increasing frequency and severity in the 3 highest dose groups.

Druckery et al. (1963a) did not observe extensive methaemoglobin formation in rats given 100 mg sodium nitrite per kg body weight in the drinking water, but there was a slight reduction in the life span. Studies by Van Logten et al. (1972) in which groups of 30 male and 30 female rats received sodium nitrite at concentrations of 0, 0.02, or 0.05% with or without glucono-δ-lactone (GDL) in the diet for 29 months did not demonstrate any significant haematological or biochemical effects. Carcinogenic action, which could be related to the administration of

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sodium nitrite with or without DEA or GDL, was not observed in either of these studies.

It has been reported that prolonged administration to rats of 1/20 of the LD_{50} of calcium and sodium nitrates disturbed the energy conversion processes such as glycolysis and the pentose phosphate cycle, changed the activity of the glutathione–ascorbic acid system in the blood and in the hepatic and cerebral tissues, raised the levels of methaemoglobin and of NADH-methaemoglobin reductase activity, and reduced the haemoglobin levels (Diskalenko & Dobrjanskaja, 1972; Diskalenko & Trofimenko, 1972).

Greenblatt & Mirvish (1972) gave 3 groups of about 40, 7–9 week old, male, strain A mice, 1 or 2 g of sodium nitrite or 12.3 g of sodium nitrate/litre of water respectively, for 20 weeks and did not observe any increase in lung tumour incidence in comparison with untreated controls. Lijinsky et al. (1973a) reported similar negative results when groups of 30 rats were given sodium nitrate at 5 g/litre or sodium nitrite at 2 g/litre in their drinking water at a daily rate of 20 ml/rat throughout most of their lifetime. Other studies by Lijinsky et al. (1973c) on groups of 30 rats given 20 ml of sodium nitrite at 2 g/litre daily, for 5 days a week, also produced negative results. Taylor & Lijinsky (1975) reported that tumour formation in 57 Sprague-Dawley rats, exposed for 2 years to a drinking solution containing sodium nitrite at 2 g/litre, was no greater than in the controls.

7.1.3 Embryotoxicity

A study has been reported by Shuval & Gruener (1972) in which 2 groups of 12 pregnant rats were given 2000 or 3000 mg of sodium nitrite per litre of drinking water, respectively. A control group did not receive any treatment. Pregnant rats developed anaemia and had higher methaemoglobin levels than nonpregnant rats receiving similar doses. There was a pronounced increase in mortality among the newborn rats of treated dams compared with those of untreated controls, particularly in the 3-week period before weaning. Mortality in the offspring was 6% in controls, 30% in those given 2000 mg/litre and 53% in those given 3000 mg/litre. Birthweights were similar in all groups but growth was markedly reduced in pups of treated dams. Such pups had thin hair coats. Treatment of 2 groups of 10 and 15 pregnant rats with 1% and 0.3%, respectively, of sodium nitrate in the diet did not result in any embryotoxic or teratogenic effects on the 9th and 10th days of gestation (Alexandrov & Jänisch, 1971).

Sleight & Atallah (1968) conducted a study in which 46 female

guineapigs, divided into 12 groups each containing at least 1 male, were given potassium nitrate in doses ranging from 300 to 10 000 mg/kg body weight in the water and potassium nitrite in amounts ranging from 300 to 10 000 mg/kg for periods ranging from 100–240 days. Reproduction in the female was grossly impaired in the high nitrate group. Fetal losses were 100% in females given 5000 or 10 000 mg/kg of the nitrite and one female died. Reproduction was maintained at lower levels of treatment. Apparently male fertility was not impaired, since conception took place at all levels of treatment. Food and water consumption and weight gains of treated animals were normal except for a diminished rate of gain at a nitrite level of 10 000 mg/kg body weight. Uterine and cervical inflammatory lesions and degenerative placental lesions were present in females in which the fetuses had been aborted, mummified, or absorbed. Sinha & Sleight (1971) reported studies in which 4 pregnant guineapigs, given sodium nitrite at 50 mg per kg of body weight, subcutaneously, underwent normal parturition. However, fetal deaths followed by abortion, occurred in 3 guineapigs given sodium nitrite at 60 mg/kg. The fetal deaths occurred approximately 1 h after nitrite administration, when the maternal and fetal methaemoglobin levels were highest. At the time of death, there were no noticeable changes in the placenta; pathological changes developed after the death of the fetuses. There were lower blood pO_2 values in the fetuses of the guineapigs treated with nitrite at 60 mg/kg than in those of the controls. Fetal death did not occur in pregnant animals given sodium nitrite at 60 mg/kg combined with simultaneous intraperitoneal treatment with 10 mg of methylene blue per kg body weight. The data suggest that fetal death resulted from hypoxia, mainly induced by maternal methaemoglobinaemia.

Studies by Shuval & Gruener (1972) indicated that methaemoglobinaemia might be induced transplacentally and that the observed limit for transplacentally-induced methaemoglobinemia was a sodium nitrite dose of 2.5 mg/kg body weight. A steep increase in effect occurred with increasing doses of sodium nitrite.

7.1.4 Mutagenicity

The mutagenic potential of nitrates and nitrites has not been studied extensively; in fact, no data are available on their mutagenic action in the mammalian systems. The mutagenicity of nitrates and nitrites with respect to the transformation of DNA has been reported (Bressler et al., 1968; Horn & Herriot, 1962; Strack et al., 1964) and nitrous acid has been shown to be mutagenic in bacterial systems such as *Escherichia coli* (Kaudewitz, 1959; Verly et al., 1967) and *Salmonella typhimurium* (de

Serres et al., 1967). Positive results in mutagenic studies have been reported with the yeast *Saccharomyces cerevisiae* (Nashed & Jabbur, 1966; Zimmerman & Schwaier, 1967; Zimmerman et al., 1966), as well as with *Aspergillus nidulans* (Siddiqi, 1962), *A. niger*, and *A. amstelodami* (Steinberg & Thom, 1940), and tobacco mosaic virus (Sehgal & Krause, 1968).

7.1.5 Interaction with nutritional factors

Studies by Kociba & Sleight (1970) showed that maternal blood levels of methaemoglobin were significantly higher in 12 ascorbic acid-deficient, pregnant guineapigs, following the subcutaneous administration of sodium nitrite at 40 mg/kg body weight, than in those on a normal diet. Following subcutaneous administration of sodium nitrite at 50 mg/kg, there was a higher percentage of fetal death in the ascorbic acid-deficient guineapigs.

Experiments were conducted by Stoewsand (1973) with young, male, guineapigs (number of animals not stated) to investigate the influence of feeding beets with naturally occurring low and high amounts of nitrates and nitrites, and the influence of dietary supplementation with ascorbic acid and methionine on methaemoglobinaemia, induced by orally administered sodium nitrite at doses of 25 or 50 mg/kg body weight. Low-nitrate beet diets seemed to "protect" guineapigs from nitrite intoxication. In addition, a 1% diet containing L-ascorbic acid and 1% methione reduced nitrite-induced methaemoglobin blood levels.

Sell & Roberts (1963) showed that *ad libitum* feeding of diets containing 0.4% potassium nitrite to 6 groups of 50 chicks depressed growth, irrespective of the amount of vitamin A administered. Also, all the test animals, except those given massive injections of vitamin A, exhibited reduced liver stores of the vitamin and enlarged thyroid glands.

Studies by Phillips (1966) demonstrated that the liver vitamin A contents of rats fed 1% potassium nitrite in diets containing carotene or vitamin A, were less than those of control rats. It was suggested that the dietary nitrite degraded the carotene and vitamin A in the digestive tract before their absorption.

7.2. N-nitroso Compounds

7.2.1 Acute and subacute toxicity studies

The acute toxicity of N-nitroso compounds is not of great toxicological significance because there is no relationship between acute toxicity and

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the carcinogenic potential of this class of compounds (Druckrey et al., 1967, Magee & Barnes, 1967). Because DMN was reported to cause cirrhosis and other toxic effects in industrial workers, Barnes & Magee (1954) examined the toxicity of this compound. Doses of 20–40 mg/kg body weight given to rats, dogs, rabbits, and guineapigs produced severe hepatic damage. A single dose of DMN given to rats orally or by intravenous, intraperitoneal, or subcutaneous injection, produced centrilobular necrosis accompanied by haemmorhages in the liver. In rats given 20 mg DMN/kg body weight the liver cells in the centrilobular and mid-zonal regions became pale and, after 18 h, the cytoplasm was amorphous and vacuolated; the nuclei were pale and irregular in outline. By 24 h, the cells were necrotic and confluent areas became haemorrhagic. Haemorrhage was usually more pronounced after 48 h but after 72 h the recovery process had begun and was almost complete in 3 weeks (Barnes & Magee, 1954; Magee & Barnes, 1962). A detailed study by light and electronmicroscopy of changes in the liver cytoplasm of rats treated with N-nitrosomorpholine has been reported by Bannasch (1968).

The acute toxicity of acyl-alkyl-nitrosamides and diazoalkanes was reported by Druckery et al. (1967), Magee & Barnes (1967), and Shank (1975). The acute toxicity (LD_{50}) of the nitroso compounds varied widely; some were only mildly toxic while others produced highly destructive lesions. N-nitroso-N-methylurethane, for example, when given orally, produced severe necrotic lesions in the stomach, congestion of the lungs, and periportal necrosis of the liver (Schmähl & Thomas, 1962; Schoental, 1960). N-nitroso-N-methylurea also produced inflammatory haemorrhagic lesions of the stomach, intestine, and pancreas and a reduction in the bone marrow when given orally to rats, (Druckrey et al., 1961, 1967).

The acute toxicity of some N-nitroso compounds, e.g. nitrosopiperidine and nitroso-morpholine, was not manifested as liver damage but as neurotoxic effects, e.g. convulsions (Lee & Lijinsky, 1966).

The acute toxicity of nitrosomines formed *in vivo* was studied by Asahina et al. (1971). Sodium nitrite was administered by gavage to mice at 100 or 150 mg/kg bodyweight alone or in combination with DMA and methylbenzylamine at doses of 500–2500 mg/kg and 800–1600 mg/kg, respectively. Combinations of the amines and nitrite produced hepatic lesions similar to those produced by DMN or nitrosomethylbenzylamine. Similar effects were noted when nitrite was administered up to 3 h after DMA but these effects were markedly reduced if the nitrite was given prior to the amine. Lijinsky & Greenblatt (1972) reported hepatic necrosis following coadministration of aminopyrine and sodium nitrite.

In a number of studies, ascorbic acid has been shown to have an inhibitory effect on: *a)* nitrosamine formation in the stomach and small intestine of rats treated with 7-chloro-2-methylamino-5-phenyl-3H-1, 4-benzodiazepine-4-oxide, (chlordiazepoxide, Librium) and sodium nitrite (Preda et al., 1976); *b)* hepatotoxicity induced by the combined administration of sodium nitrite and aminopyrine in rats (Kamm et al., 1973) and mice (Greenblatt, 1973); *c)* liver necrosis produced by DMA and nitrite administered to rats by gavage (Cardesa et al., 1974); *d)* the teratogenic and transplacental.carcinogenic effects produced in rats by treatment with alkylurea and nitrite (Ivankovič et al., 1973); and *e)* the induction of lung adenomas in mice by prolonged treatment with morpholine, piperazine, and methylurea plus nitrite (Mirvish et al., 1975).

Species differences exist with respect to the toxic effects of *N*-nitroso compounds. Mink appear to be especially sensitive to DMN, and as in other species, the effects are seen primarily in the liver. Carter et al. (1969) noted widespread liver degeneration and necrosis of hepatocytes in mink given DMN at 2.5 or 5.0 mg/kg in the diet for 7–11 days. The liver lesions were accompanied by bile-duct proliferation, ascites, and haemorrhage of the gastrointestinal tract. Sheep and cattle are also more sensitive to nitrosamines than laboratory animals (Sakshaug et al., 1965; Koppang, 1964). Sheep, given a single dose of DMN at 5 mg/kg body weight or 12 doses of 0.5 mg/kg, died or were severely affected displaying anoxia, lack of rumination, ataxia, and respiratory difficulties, while cattle given DMN at 0.1 mg/kg body weight showed pronounced hepatotoxic effects in 1–6 months.

Pathological and biochemical effects, observed in the liver of a number of animal species including the rat following continuous administration of nitrosamides, have been reviewed by Magee & Barnes (1967) and Magee et al. (1976); the main effect is inhibition of protein synthesis which might be a result of an accelerated breakdown of messenger ribonucleic acid (RNA).

7.2.2 Carcinogenicity

The carcinogenic activity of *N*-nitroso compounds has been summarized in several reviews (Druckrey, et al., 1967; Magee & Barnes, 1967; Magee et al., 1976). Various animal species including mammals, birds, fish, and amphibia have been shown to be susceptible to the carcinogenic action of nitrosamines. At present, some 80 nitrosamines and 23 nitrosamides have been tested in rats and about 80% of the nitrosamines and practically all the nitrosamides have proved to be

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carcinogenic (Montesano & Bartch, 1976). These carcinogens show a marked organ specificity as shown in Table 3.

Table 3. Localization of tumours induced by N-nitroso compounds in rats[a]

Target organ	Number of N-nitroso compounds affecting target organ	
	Nitrosamines	Nitrosamides
liver	35	2
oesophagus-pharynx	32	3
nasal cavities	18	–
respiratory tract	10	1
kidney	8	9
tongue	8	–
forestomach	7	11
bladder	4	1
central and peripheral nervous system	2	9
ear duct	2	1
testis	1	–
ovary	1	2
mammary glands	1	1
sites of injection	3	4
intestine	–	7
glandular stomach	–	6
skin	–	3
jaw	–	1
uterus	–	2
vagina	–	1
haemopoietic system	–	2

[a] From: Montesano & Bartch (1976)

Nitrosamines produce a carcinogenic effect in the liver, oesophagus, respiratory system, and kidney, whereas nitrosamides affect the peripheral and central nervous systems, and the gastro-intestinal tract organs.

The dose schedule seems to play an important role in this organ specificity. For example, in rats, long-term exposure to relatively low doses of DMN induced mainly liver tumours, whereas a single or a few high doses over a short period induced mainly kidney tumours (Magee & Barnes, 1962). Similar responses in relation to the liver and oesophagus were observed with DEN in rats (Druckey et al., 1967). The route of administration does not seem to play an important role in the carcinogenicity of this chemical. It is worthwhile pointing out that many of these chemicals were carcinogenic following a single administration and that, furthermore, exposure of rats to a single dose during pregnancy

resulted in carcinogenesis in the immediate descendants and also in the two succeeding generations (Tomatis et al., 1977).

Nitrosamines exert their adverse biological effects after being metabolically activated by microsomal mixed function oxidases to form reactive intermediates. On the other hand, nitrosamides decompose enzymatically to reactive, and in most cases, alkylating derivatives. The importance of hydroxilation of the α-hydrogen atoms of the nitrosamines is demonstrated by the lack of carcinogenicity of compounds, such as diphenylnitrosamine, which do not have this α-hydrogen (Magee et al., 1976).

7.2.2.1 *Interspecies variation in response*

Several *N*-nitroso compounds have been tested in different animal species. DEN, tested in more than 20 species including primates, induced tumours of the liver in all of them, together with various tumours of other organs. In some cases, there have been marked differences between species in response to *N*-nitroso compounds. For example, nitrosoheptamethyleneimine produced squamous carcinoma of the lung in rats (Lijinsky et al., 1969), the same effects in European hamsters, but tumours of the forestomach and the lung in Syrian hamsters (Lijinsky et al., 1970). In all three species, this compound also induced oesophageal tumours. Nitrosomethylurethane induced tumours of the pancreas in guineapigs (Druckrey et al., 1968) and forestomach carcinomas in rats. Bis-2-hydroxypropyl-nitrosamine produced pancreatic tumours in Syrian hamsters. These results show the difficulty of attributing, with certainty, any particular tumour response of man to a particular *N*-nitroso compound.

7.2.2.2 *Intraspecies variation in response*

Kuwahra et al. (1972) administered DMN orally or by subcutaneous or intraperitoneal injections, to 8 to 12-week-old, male and female mice of the DDD, BALB/C, and SJL/J strains. Tumours were found mainly in the retroperitoneum and abdominal cavity and the incidence and distribution were little affected by the strain of mouse but markedly by the route of administration. Clapp et al. (1971) noted that DEN induced forestomach and oesophageal squamous cell carcinomas in both BALB/C and RF/Un strains of mice but induced liver haemangiosarcomas in the former and hepatomas in the latter. In addition, DEN induced a high incidence of lung tumours in RF mice but a very low incidence in BALB/C mice. In both strains, DMN induced lung adenomas and liver haemangiosarcomas. Tissue sensitivity did not appear to be related to the spontaneous tumour incidence of the strain.

7.2.2.3 *Dose-response relationships of* N-*nitroso compounds*

Druckrey et al. (1963b) were the first to study the dose-response relationships of *N*-nitroso compounds in relation to minimum effect doses. They concluded, from studies in rats given DEN, that the carcinogenic effect was related to dose and induction time in such a way that $d.t^{2.3} =$ constant, where d represents the daily dose and t the induction time. Even a low dose of 0.15 mg/kg body weight resulted in liver carcinomas in 27 out of 30 surviving animals. The average induction time was 609 ± 38 days. The lowest dose studied (0.075 mg/kg body weight) produced liver and oesophageal tumours in all four surviving animals with an induction time of 830 days.

Mohr & Hilfrich (1972) gave a single intravenous injection of DEN to rats at 8 dose levels between 1.25 and 160 mg/kg body weight. A dose-response relationship was noted; at the lowest dose of 1.25 mg/kg, only one kidney tumour was observed in 20 treated rats, but, at higher doses, the incidence of these tumours increased. Single dose experiments with ethylnitrosourea in a transplacental carcinogenesis experiment also showed that the incidence of induced tumours (mainly of the brain and nervous system in the progeny) was directly proportional to the dose of the carcinogen. Four doses between 1 and 50 mg/kg were used and even in the lowest dose group 36/41 animals in the progeny died with tumours (Swenberg et al., 1972). Terracini et al. (1967) fed rats concentrations of DMN ranging from 2 to 50 mg/kg diet. At 2 and 5 mg/kg the incidence of liver tumours in the survivors was 1/26 and 8/74, respectively, at 60 weeks. At 20 and 50 mg/kg, liver tumours were observed in more than 60% of the test animals. From this study, it can be concluded that a dietary level of DMN of 5 mg/kg is still carcinogenic in the rat.

A current dose-response study on rats receiving oral doses of *N*-nitrosopyrrolidine has been reported by Preussmann (unpublished data).[a] Liver tumours were induced in groups receiving 10, 3, and 1 mg/kg body weight per day, respectively, but not in a group receiving 0.3 mg/kg per day.

Clapp & Toya (1970) gave male, RF mice DMN in the drinking water for various lengths of time, with cumulative doses ranging from 87 to 243 mg/kg body weight. The incidence of lung adenomas in all treated groups was higher than that in the untreated control groups. Whereas, apparently, the incidence of hepatocellular tumours was not affected, the incidence of liver haemangiosarcomas increased in the higher dose groups and reached a maximum of 96%. Bertram & Craig (1973) using 2

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[a] Paper presented at the Proceedings of the Second International Symposium on Nitrite in Meat Products, Zeist, September 1976.

groups of 100, C57BL/6 mice found that the incidence of bladder tumours fell from 80% in animals given 30 mg of nitrosodi-N-butylamine per kg/body weight per day to 36% at 7.5 mg/kg body weight per day. Sander & Schweinsberg (1973) noted that an increasing incidence of tumours of the oesophagus and forestomach was induced in NMRI mice by adding a given dose of methylbenzylnitrosamine to the drinking water at various times to provide total doses ranging from 1.4 to 44 mg/kg body weight.

In studies conducted by Vesselinovitch (1969), DMN was administered repeatedly by intraperitoneal injection to 73 male and 54 female C57BL × C3H mice starting at 7 days of age. The injections were given at 3 day intervals for a total of six doses of 1, 2, or 4 mg/kg body weight. Mice killed at 66 weeks of age showed an increase in the incidence of hepatomas, hepatocarcinomas, lung adenomas, and haemangiomas as the dose increased. The incidence of liver tumours was higher in males (75%) than in females (28%).

Tomatis & Cefis (1967) gave a dose of 3.6 mg of DMN by stomach tube to one group of 30 Syrian golden hamsters in 3 administrations of 1.6 mg, 1.0 mg, and 1.0 mg over a 5-week period. A second group received a single dose of 1.6 mg. Both dosing regimes produced liver cell carcinomas and a few cholangiomatous lesions but no kidney tumours. Montesano & Saffiotti (1968) administered 12, weekly, subcutaneous injections of DEN at 0.5, 1.0, 2.0, or 4.0 mg to 4 groups of 36 Syrian golden hamsters. The results demonstrated a positive dose-response relationship for tumour induction in the upper respiratory tract (nasal cavities, larynx, and trachea). The incidence of nasal cavity tumours, which developed early, varied from 6/35 to 27/36. The incidence of tumours of the larynx varied from 6/35 to 26/36 and that of tumours of the trachea from 29/35 to 35/36.

7.2.2.4 *Tumour induction by combined administration of nitrites and amines or amides*

Carcinogenic effects following the combined administration of secondary and tertiary amines or amides with nitrite have been reported. Tumours were not observed when sodium nitrite or the amine or amide were given singly. Tumours of the same type occurred at the same site when the N-nitroso compound, believed to be formed from the nitrite and amine or amide, was given as a positive control. In some of the experiments, the nitrite was given in the drinking water and the amine or amide in the food. In others, the compounds were combined in the same medium (drinking water or food).

Preussman (1975) reviewed studies in which tumours that had been

produced at certain sites by the oral administration of combinations of amines and nitrite, were compared with the tumours induced by the corresponding *N*-nitroso compounds. Table 4 has been adapted from Preussman (1975) and updated.

Table 4. *In vivo* formation of *N*-nitroso compounds following oral administration of sodium nitrite and amino compounds as demonstrated by specific carcinogenesis[a]

sodium nitrite + amine oral administration amino compounds	Observed tumour site	Expected tumour site for corresponding *N*-nitroso compound	Reference
Rat			
diethylamine	—	liver	Druckrey et al. (1963b) Sander et al. (1968) Sander (1971a)
triethylamine	—	liver (for diethylnitrosamine)	Schweinsberg & Sander (1972)
morpholine	liver, kidney, lung	liver	Sander & Bürkle (1969), Sander (1971c)
N-methylbenzylamine	oesophagus	oesophagus	
piperidine	..	oesophagus liver	
N-methylanaline	oesophagus, nasal cavity	oesophagus	
N-methylcyclohexyl-amine	oesophagus	oesophagus	Sander (1971a)
N-methylbenzylamine	oesophagus	oesophagus	
phenylbenzylamine	negative	untested	
N,N-dibenzylethylene-diamine	negative	untested	
indole	negative	untested	
morpholine	liver (kidney)	liver (kidney)	Shank & Newberne (1972)
aminopyrine (Pyramidon)	liver	liver (for dimethyl-nitrosamine)	Lijinsky et al. (1973c)
heptamethyleneamine	lung oesophagus	lung oesophagus	
aminopyrine	liver (for DMN)	liver	Taylor & Lijinsky (1975)
oxytetracycline	liver (for DMN)	liver	Greenblatt et al. (1973)
proline	negative	untested	
hydroxyproline	negative	untested	
arginine	negative	untested	
N-methylacetamide	negative	forestomach	
N-methylurethane	negative	forestomach	
N-ethylurethane	negative	(carcinogenic following i.v. administration)	Sander (1971b)
acetanilide	negative	untested	
glycylglycine	negative	untested	
6-methyluracil	negative	untested	
N-methylguanidine	negative	untested	
N-phenylurea	negative	(carcinogenic following s.c. administration)	Sander (1971b)
N-methylthiourea	negative	untested	
N-methylurea	brain, nervous system, kidney	brain, nervous system, kidney	
N-ethylurea	brain, nervous system, kidney	brain, nervous system, kidney	

sodium nitrite + amine oral administration amino compounds	Observed tumour site	Expected tumour site for corresponding N-nitroso compound	Reference
N'N'-dimethylurea	brain, nervous system, kidney	brain, nervous system, kidney	Sander (1971b)
imidazolidinone	kidney	(carcinogenic following s.c. administration)	Sander & Bürkle (1971)
ethylurea (during pregnancy, trans-placental)	brain, nervous system (in descendants)	brain, nervous system (in descendants)	Ivanković & Preussmann (1970); Osske et al. (1972)
Mouse			
dimethylamine	lung (adenoma)		
piperazine	lung (adenoma)	lung (adenoma)	Greenblatt
morpholine	lung (adenoma)	lung (adenoma)	et al. (1971)
N-methylaniline	lung (adenoma)	lung (adenoma)	
morpholine	lung (adenoma)	lung (adenoma)	
N-methylbenzylamine	oesophagus forestomach	oesophagus forestomach	Sander (1971a)
piperazine	lung (adenoma)	lung (adenoma)	Greenblatt & Mirvish (1972)
N-methylurea	lung (adenoma)	lung (adenoma)	Mirvish et al.
N-ethylurea	lung (adenoma)	lung (adenoma)	(1973b)

7.2.2.5 *Dose-response relationship for combinations of nitrite and amines*

Greenblatt & Mirvish (1972) conducted a study in which groups of 40, male, strain A mice, given 0.69–18.75 g of piperazine per kg of food and 0.05–2.0 g of sodium nitrite per litre of drinking water for 20–25 weeks, were killed 10–13 weeks later. The yield of lung adenomas was statistically significantly greater than in untreated controls, when doses as low as 0.69 g piperazine/kg plus 1.0 g sodium nitrite/litre, or 6.25 g piperazine/kg plus 0.25 g sodium nitrite/litre were given. In the animals given 6.25 g piperazine per kg of food, plus 2.0 g sodium nitrite per litre of water, 39 out of 40 had adenomas, in contrast with 5 out of 39 controls. A progressive decrease in the incidence of adenomas was seen with reduction in the nitrite level. On the other hand, when the nitrite level was maintained at 1.0 g/litre, the tumour incidence increased marginally when the dose of piperazine was increased from 0.69 g to 18.75 g/kg of food. Nitrite and piperazine administered alone yielded negative results.

When various concentrations of nitrite and morpholine (up to 1000 mg/kg) were fed to groups of Sprague-Dawley rats, the development of hepatocellular carcinomas and angiosarcomas identical to those produced by N-nitrosomorpholine was noted (Newberne & Shank, 1973).

A 2–3% incidence of hepatomas was induced with only 5 mg of morpholine plus 1000 mg of sodium nitrite per kg of food, or 1000 mg of morpholine plus 50 mg sodium nitrite/kg. The tumour incidence was

98% when the concentration of both components was 1000 mg/kg, and 0% when diet alone was given.

7.2.2.6   *Transplacental carcinogenesis*

Induction of neoplasms in offspring as a result of prenatal exposure to various N-nitroso compounds and related substances has been reported in different animal species including the rat, mouse, golden hamster, guineapig, rabbit, dog, and monkey (Magee et al., 1976). The various routes of administration (subcutaneous, intraperitoneal, intravenous, oral, and inhalation) were equally effective. However, a critical factor was the time of treatment during gestation.

Since many of these substances appeared to be metabolically activated to exert their carcinogenic action, the lack of an adequate metabolic system during the first period of pregnancy may explain the failure to observe tumours, when exposure was limited to this period. DMN induced tumours in the offspring only when administered during the last days of pregnancy (Alexandrov, 1968a). The data of Magee (1972) are in keeping with these findings; the formation of 7-methylguanine in the nucleic acids of fetal rat tissues was detected following treatment with DMN on the 21st day of pregnancy but not on the 15th day.

When considering the effect of different acyl alkyl nitrosamides, N-ethyl-N-nitrosourea was found to be more active than its methyl analogue (Alexandrov, 1969b; Ivanković & Druckrey, 1968).

The sensitivity of the nervous system at various stages of prenatal development was examined in rats and golden hamsters by Ivanković & Druckrey (1968) by means of single intravenous injections of N-ethyl-N-nitrosourea on different days during gestation. A high incidence of tumours was observed after treatment on the 18th day or shortly before delivery (21st day) but none developed when the mother animals were treated before the 12th day. A positive dose-response relationship was obtained with a single dose in the range of 5 to 80 mg/kg bodyweight on the 15th day. A single dose as low as 2 mg/kg was sufficient to produce malignant, neurogenic tumours in 2/25 newborn whereas in adult animals an effect was produced in 50% of the animals by a single dose at 160 mg/kg. Thus a 50-times higher sensitivity was demonstrated for the fetal nervous system. Similar results were obtained by Swenberg et al. (1972) in Sprague-Dawley and Fisher rats using a dose of N-ethyl-N-nitrosourea as low as 1 mg/kg. In a recent study on rats, Maekawa & Odashima (1975) explored the effects of subcutaneous injections of N-butyl-1-nitrosourea on embryonal, fetal, and newborn nervous systems. Treatment during early gestation led only to the death of the embryo. When the compound was given in the middle of the

pregnancy (8–14 days), a high incidence of nervous system tumours and pituitary tumours was found in the offspring. Treatment late in pregnancy (15–21 days) led to the development of nervous system tumours in 33/36 offspring.

When lactating animals were given the compounds, tumours were induced in 19/39 of the offspring, the target organs being mainly the testes and uterus.

Tomatis (1977) reported an increased incidence of cancer in the descendants (second and third generation) of transplacentally treated rats. In certain cases, the tumour-producing doses were lower than those for adult animals. The target organs were usually similar in fetal, newborn, and adult animals but the nervous system has been shown to be highly sensitive to certain compounds, notably the nitrosoureas. The induction time for transplacental tumours may be shorter than that in adults.

7.2.2.7 *Morphological studies*

Morphological events associated with the development of hepatic tumours, following exposure to *N*-nitroso compounds, were studied by Rabes et al. (1970), Scherer et al. (1972), and Schmitz-Moorman et al. (1972). Schmitz-Moorman et al. (1972) followed the carcinogenesis induced by DMN in rat liver, using histological and histochemical procedures. In the initial phase, there were vacuolar changes accompanied by a decrease in the RNA and glycogen contents. In the second phase, glycogen storage was noted while in the third phase, basophilic cells with atypical nuclei were observed and could not be distinguished from microcarcinomas. The RNA content of these cells was substantially higher. The activities of acid phosphatase (3.1.3.2), succinic dehydrogenase (1.3.99.1) and glucose-6-phosphatase (3.1.3.9) substantially decreased. In the last carcinogenic stage, dramatic changes were noted in several enzymes (Jennissen et al., 1971).

Early changes in the lung tissue of mice exposed to carcinogenic doses of DMN were reported by Calafat et al. (1970) and DEN-induced alterations in the respiratory tract of hamsters were reported by Althoff et al. (1971). Greenblatt & Rijhsinghani (1969) compared the cytopathological changes induced by DEN and DMN in the nasal epithelium of hamsters; other light microscopic studies have been published by Bertram & Craig (1972), Boyland et al. (1968), Hicks et al. (1973), and Terracini et al., (1967). The histological appearance of tumours under the electron microscope has been described by Kirkland & Pick (1973). Veno-occlusive disease in the liver of rats given DMN was

reported by Butler & Hard (1971) who had also studied the effects of this compound on rat testes (Hard & Butler, 1970c).

Other studies, by light microscopy, to observe early changes related to the formation of neoplasms induced in the kidneys of rats treated with DMN, were reported by Benemanski & Litvinov (1969), Hard & Butler (1970a, 1970b), and Hard et al. (1971).

Electron microscopic studies of DMN-induced liver and kidney tumours have been reported in rats (Bhathal & Hurley, 1973; Geil et al., 1968; Hard & Butler, 1971a, 1971b, 1971c; Ireton et al., 1972; Jasmin & Cha, 1969; Svoboda & Higginson, 1968) and in mice (Takayama, 1968). Treatment with DEN resulted in ultrastructural changes in the lungs of hamsters (Stracks & Feron, 1973) and in the liver of monkeys (Williams, 1970) and rats (Bader et al., 1971; Bruni, 1973). Early morphological changes in the bladder of rats exposed to N-butyl-N-butanol-4-nitrosamine were reported by Riedel & Piper (1973).

7.2.2.8 *Biochemical mechanisms*

Extensive work on the biochemical mechanisms of carcinogenesis produced by N-nitroso compounds has been reviewed by Magee & Barnes (1967) and more recently by Magee et al. (1976). Alkylation of nucleic acids by N-nitroso compounds or their metabolites has been investigated extensively and has been suggested as the mechanism of carcinogenicity (Krüger, 1972, 1973; Lijinsky et al., 1973b; Swann & Magee, 1971; Takayama & Muramatsu, 1969). In early studies, it was thought that the 7 position of guanine was the significant site of the reaction.

Following the important discovery by Loveless and Hampton (1969) of the O,6-alkylation of deoxyguanosine by nitrosomethylurea (NMU) and the implications of this reaction in terms of carcinogenicity, O'Connor et al. (1973) estimated the amount of methylation at the O,6-position of guanine DNA isolated from animals treated with DMN. This base accounted for 4–6% of the methylation after DMN treatment. O,6-methylguanine was lost (by "excision") from DNA with a half life of approximately 13 h. The excision of the abnormal components of DNA, O,6-methylguanine, and the unstable acid-labile products, may be important processes in liver carcinogenesis. O'Connor et al. (1973) suggested that events leading to the development of tumours may be related to the efficiency of the cellular excision system for certain products of alkylation rather than to the level of alkylation obtained at a particular site.

7.2.2.9 *Interaction with various chemical factors*

Several studies are available on the combined effects of N-nitroso compounds and other carcinogens. Schmähl et al. (1963) showed that

combined oral administration of the hepatocarcinogens DEN and *N,N*-dimethyl-4-(phenylazo)benzenamine (4-dimethyl-aminoazobenzene) significantly reduced the time for tumour induction and that only 66% of the total dose administered when single compounds were used, was necessary for tumour induction. Takayama & Imaizumi (1969) also demonstrated synergism with the combined administration of DMN and 4-dimethylaminoazobenzene. Liver tumours were induced in rats by sequential administration of the two carcinogens in doses that did not induce tumours when each carcinogen was given alone and the tumour induction time was reduced. The syncarcinogenic effects of a single dose of a combination of DEN and carbon tetrachloride were observed by both Pound et al. (1973) and Schmähl et al. (1965). The incidence of liver cancer increased and the induction time decreased in combined treatments compared with treatment with the individual compounds. Schmähl (1970) administered hepatocarcinogens (DMN, DEN, nitrosomorpholine, and 4-dimethylaminoazobenzene) to rats in such low daily doses that the administration of any one of the compounds alone did not lead to tumours during the lifetime of the animals. However, combined administration induced tumours in 43% of the treated animals. Combined administration to rats of DMN plus 1,2-dihydro-3-methylbenz[j]aceanthrylene (3-methylcholanthrene) did not increase the number of liver tumours compared with that induced by DMN alone but resulted in tumours in the lungs, that were not seen in the treatment with DMN alone (Hoch–Ligeti et al., 1968).

1-Isothiocyanatonaphthalene (1-napthyl-isothiocyanate) and 3-methylcholanthrene failed to inhibit the hepatocarcinogenic effects of DEN (Makiura et al., 1973). Similarly, local treatment of the glandular mucosa of the stomach of rats with 4-nitroquinoline-1-oxide or *p*-dimethylaminoazobenzene did not have any effect on the production of liver or oesophagal tumours due to oral administration of DEN (Odashima, 1969). It has been reported that ionizing radiation did not increase tumour incidence in animals exposed to nitrosamines (Flaks et al., 1973; Schmähl et al., 1966).

The effect of many other substances on nitrosamine carcinogenesis has been studied. For example, various dusts including aluminium (III) oxide, magnesium (II) oxide, and carbon had little effect on the respiratory carcinogenesis of DEN in hamsters (Stenback et al., 1973) but other studies on hamsters demonstrated that iron (III) oxide markedly increased the incidence of respiratory tract tumours induced by DEN (Feron et al., 1972). The synergistic effects of various substances on respiratory carcinogenesis in hamsters given *N*-nitroso compounds were reviewed by Montesano (1970).

The effect of noncarcinogenic chemicals on the incidence of other

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tumours induced by *N*-nitroso compounds has also been studied. In rats given DEN, liver tumour incidence was decreased by the administration of calcium heparin (Platt & Hering, 1973), aminoacetonitrile (Hadjiolov, 1971), and reserpine (Lacassagne et al., 1968). Lactoflavin, nicotinamide, or 2,6-bis-(diethanolamino)-4,8-dipiperidino-pyrimido [5,4-d] pyrimidine (dipyridamole) (Schmähl & Stackelberg, 1968) and hydrocortisone (Schmähl et al., 1971) did not influence the incidence of liver tumours induced in rats by DEN. Tryptophan was shown to inhibit the production of liver tumours, but not bladder tumours in rats exposed to *N*-nitrosodibutylamine (Okajima et al., 1971). *N*-(2-chloroethyl)-*N* (phenylmethyl) benzenemethanamine (dibenamine) did not have any effect on the incidence of oral, pharyngeal, or oesophageal tumours in DEN-treated rats, but did significantly reduce the number and severity of hepatic neoplasms (Weisburger et al., 1974). In mice, treatment with phenobarbital decreased the toxicity and carcinogenicity of DEN (Kunz et al., 1969).

7.2.2.10 *Miscellaneous modifying factors*

Nasal infection of mice with the influenza viruses PR8/FIK and A2/Bethesda 10/63 followed by treatment with DEN significantly increased lung tumour incidence in comparison with that in mice treated only with DEN (Schmidt-Ruppin & Papadoupulu, 1972). The Motol virus has been shown to increase hepatic carcinoma in mice given DEN (Kordač et al., 1969). Rats with chronic respiratory disease showed an increased lung tumour incidence when given nitrosoheptamethylenemine compared with germ-free or specific pathogen-free animals (Schreiber et al., 1972).

Feeding with a protein-deficient diet protected rats against the lethal and hepatotoxic effects of DMN (McLean & Verschuuren, 1969) but increased the incidence of renal carcinomas (McLean & Magee, 1970). Low protein diets and low zinc intake failed to influence the incidence of oesophageal tumours in rats treated with *N*-methyl-*N*-nitroso-'pentyl' amine (Van Rensburg, 1972). A single intraperitoneal injection of DMA induced nasal tumours in rats, previously starved for 48 h (Noronha & Goodall, 1972).

The carcinogenic activity of nitroso-*N*-(hydroxybutyl) butanamine (butyl-(*N*-hydroxybutyl) nitrosamine) appears to be influenced by sex hormones (Bertram & Craig, 1972). Tumours developed much earlier in male, than in female mice. This difference was abolished, however, if the males were castrated or, conversely, if the females were treated with testosterone.

Surgical manipulation of experimental animals may influence the induction of tumours by *N*-nitroso compounds. This has been shown for

partial hepatectomy (Craddock, 1971; Grunthal et al., 1970; Rabes et al., 1971) unilateral nephrectomy (Ito et al., 1969) and ureter ligation (Ito et al., 1971).

7.2.3 Embryotoxicity and teratogenicity

Whereas nitrosamines are reported to have toxic and lethal effects on the embryo, usually at dose levels that are toxic to the mother animals, nitrosamides (N-ethyl -and N-methyl urea) bring about malformations of several of the organs and systems of the developing organisms at levels not toxic to the pregnant animal. Animal experiments have shown that immature tissues are especially sensitive to these compounds. Dose-response relationships have been established and the existence of a noneffect level has been indicated.

DMN, administered orally at 30 mg/kg to pregnant rats, produced increased prenatal mortality (Alexandrov, 1967). Toxic effects on the embryo were noted following intravenous or intraperitoneal administration of DMN at various times during gestation but no teratological abnormalities were observed. Similar results were observed with DEN and with nitroso-N,N-bis butanamine (nitrosodi-N-butylamine) in rats (Alexandrov 1967), and with DEN in hamsters (Pielsticker, 1967). The aromatic nitroso compounds such as nitrosomethylbenzenamine (nitrosomethylaniline) were toxic to the embryo and had a mild teratogenic action when given to rats at the maximum tolerated doses (Alexandrov, 1968a, 1968b).

Treatment of pregnant rats with a single dose of 10–30 mg of nitrosomethylurea per kg body weight on day 9 of gestation produced anophthalmia, hydrocephaly, exencephaly, and occasionally spina bifida while treatment on days 12–15 produced microcephaly (Alexandrov, 1969a; Koyama et al., 1970; Napalkov, 1971; Von Kreybig, 1965a, 1965b; Von Kreybig & Schmidt, 1966, 1967).

The teratogenic action of nitrosoethylurea in rats was described by Druckrey et al. (1966) and Ivanković & Druckrey (1968) who noted that oligodactylia and syndactylia of the fore and hind limbs were dose-related. Von Kreybig & Schmidt (1967) and von Kreybig (1968) confirmed these effects and also found brain anomalies. Napalkov (1971) and Wechsler (1970, 1971) reported similar results. The teratogenic and embryotoxic effects of N-nitroso compounds including the pathogenesis of brain lesions were reviewed by Wechsler (1973).

7.2.4 Mutagenicity

The mutagenicity of N-nitroso compounds was reviewed by Magee & Barnes (1967) and more recently by Montesano & Bartch (1976). Until

recently, the data available were mainly concerned with the nitros-amides, the data on nitrosamines being limited to test systems which do not take into consideration the metabolic activation of these compounds by mammalian enzymes. As with other biological effects, there is a clear distinction between the mutagenic action of nitrosamides and nitro-samines. Nitrosamides were found to be mutagenic to almost all genetic indicators; this was attributed to the nonenzymatic formation of alkylating reactants.

On the other hand, nitrosamines have been reported, prior to 1970, to have a much more limited range of mutagenic activity; they were found to be mutagenic in tests with *Drosophila melanogaster* (Pasternak, 1964) but no such activity was observed in assays in which bacteria, yeast, or fungi were used. Malling (1966) showed that DMN and DEN were mutagenic for *Neurospora crassa* when the conidia were suspended in Udenfriend's hydroxylating model system and the treatment carried out under conditions believed to give rise to the same metabolic products as those formed by the action of liver enzymes in rat and mouse. Garbidge & Legator (1969), using the host-mediated assay, were the first to show that DMN, administered to mice, induced mutations in *Salmonella typhimurium*, which had been injected beforehand and was then re-isolated from the peritoneal cavity. Most of the mutagenicity assays were carried out using bacteria or fungi as genetic indicators in the presence or absence of a microsomal activation system. Only a few data are available on the mutagenicity of DMN in mammalian systems such as the dominant lethal test or cytogenetic studies. However, in general, the last two systems have a very low sensitivity and false negatives can easily result with *N*-nitroso compounds. The early observation of Pasternak (1962) that DMN induced lethal mutations in *Drosophila melanogaster* demonstrated the possible value of this system for testing the mutagenicity of compounds that require metabolic activation.

The mutagenicity of certain nitrosated pesticides, herbicides, and primary amines has been examined. The *N*-nitrosated derivatives of the pesticides propoxur and carbaryl, which are aryl-*N*-methyl carbamates, and of the herbicide benzothiazuron, a methylurea derivative, induced mitotic gene conversion in *Saccharomyces cerevisiae* (Sibert & Eisen-brand, 1974). *N*-nitrosocarbaryl was also found to be mutagenic in *Haemophilus influenzae* (Elespuru et al., 1974). Endo et al. (1973) screened a number of *N*-nitrosated guanidine derivatives for their ability to induce base pair substitutions in *Salmonella typhimurium* and a muta-genic effect was observed for many of these compounds; nitrosated methylguanidine was the most potent.

The mutagenicity of sodium nitrite, DMA, methylurea, and ethylurea

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given orally to mice, has been demonstrated in a host-mediated assay using a strain of *Salmonella typhimurium* as an indicator (Couch & Friedman, 1975). When combined with sodium nitrite, both ethylurea and methylurea had a greater effect than DMA.

Other properties of *N*-nitroso compounds such as cell transformation *in vitro* and their influence on DNA repair mechanisms are being investigated, but as yet, there are not sufficient data for their evaluation.

8. EFFECTS OF NITRATES, NITRITES AND *N*-NITROSO COMPOUNDS ON MAN

8.1 Nitrates and Nitrites

In the erythrocytes of healthy individuals, the process of methaemoglobin formation and reduction is continuous. The mean content of methaemoglobin in healthy populations is usually reported to be below 2% of the total haemoglobin concentration (Committee on Nitrate Accumulation, 1972; Gobbi et al., 1974; Smith, 1972). However, Goldsmith et al. (1975) recently found mean levels in Californian populations ranging up to 2.11% with 1% of the adults and 8% of infants having methaemoglobin levels exceeding 4%. Higher values are found in premature than in full-term infants and levels in infants are higher than those in older children and adults (Kravitz et al., 1956). At a level of about 10%, methaemoglobinaemia may produce symptomless cyanosis, whereas levels of 20–50% are associated with conspicuous cyanosis accompanied by hypoxic signs and symptoms, such as weakness, exertional dyspnoea, headaches, tachycardia, and loss of consciousness (Arena, 1970; Committee of Nitrate Accumulation, 1972; Jaffé & Heller, 1964). The lethal concentration of methaemoglobin is not known, but death may occur at levels exceeding 50% (Committee of Nitrate Accumulation, 1972).

8.1.1 Epidemiological studies

The National Academy of Sciences, USA (Committee on Nitrate Accumulation, 1972) recently reviewed about 350 cases of methaemoglobinaemia in the USA and about 1000 cases in Europe that were reported to be associated with the intake of nitrates in well water or in food. There were 41 fatalities in the USA and about 80 in Europe. Only one case of infant methaemoglobinaemia resulting from the consumption

of water from a municipal water supply was reported in the USA (Vigil et al., 1965).

8.1.1.1   *Exposure through water*

The toxicity to man of nitrates in water was first reported by Comly (1945). He noted high levels of methaemoglobin and the associated signs of nitrate toxicity in 2 infants who had consumed water containing high concentrations of nitrates (619 and 388 mg/litre). Since then, several epidemiological and case studies have been carried out in various parts of the world, particularly in areas with naturally high nitrate levels in water.

Robertson & Riddell (1949) reported 10 cases in which infants receiving powdered milk preparations made with well waters with nitrate concentrations exceeding 75 mg/litre had blood levels of me-thaemoglobin ranging from 5 to 50% of total haemoglobin. Two of the infants, whose dried milk preparations had been reconstituted with well waters containing nitrate levels of 1200–1300 mg/litre, had methae-moglobin levels of 25% and 44% of total haemoglobin, respectively; both cyanosed rapidly and died before therapy could be applied.

In the state of Minnesota (USA), Bosch et al. (1950) reported 139 cases of infant methaemoglobinaemia due to the ingestion of well water with a high nitrate content (over 89 mg/litre); the mortality rate was 10%. In Kansas, 13 cases of infant methaemoglobinaemia including 3 deaths caused by drinking well water, were reported between the early 1940s and 1950 (Walton, 1951). Many other cases in the USA have been reported by this author. On the other hand, infant methaemoglo-binaemia was absent in urban areas of New York and the province of Ontario, Canda where nitrate levels in water were low (Ciavaglia & Thompson, 1969). Knotek & Schmidt (1964) reported 115 cases of methaemoglobinaemia from a total of 5800 children born in central Czechoslovakia between 1953 and 1960. Of these, 8% were fatal, 52% were severe, and 40% were mild. Most deaths were associated with the consumption of water containing nitrate concentrations ranging from 70 to 250 mg/litre. In these cases, methaemoglobinaemia was invariably associated with the consumption of infant milk preparations, which contained microorganisms such as *B. subtilis*, capable of reducing nitrates to nitrites. These workers reported the inhibitory effect of buttermilk on this conversion. They attributed this effect to the presence of *Streptococcus lactis* which produces the antibiotic nisin and is capable of preventing the growth of the spores of *B. subtilis*.

Shuval & Gruener (1972) studied communities with various concentrations of nitrates in the drinking water, in Israel. Infants from rural

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communities with reported nitrate levels of 50 – 90 mg/litre in the water supplies were compared with controls where the average level was 5 mg/litre. They did not find any definite cases of methaemoglobinaemia nor were there any significant differences in the methaemoglobin levels. However, there was widespread consumption of citrus juices and the water consumption in infant milk preparations was low (94% of the infants studied were breast fed or received whole cow's milk). Soviet literature contains information concerning a comparatively small number of cases of symptomless methaemoglobinaemia caused by nitrates in water (Diskalenko, 1969; Motylev, 1969). In children whose drinking water contained a high level of nitrates, the methaemoglobin level did not usually exceed 10% although higher levels were found occasionally. Sattelmacher (1962) and Simon et al. (1964) compiled 1060 and 745 cases, respectively, of infant methaemoglobinaemia due to nitrate-contaminated water in the Federal Republic of Germany. Most of the cases were associated with water from private wells and in 84–90% of the cases the water contained nitrate concentrations exceeding 100 mg/litre (although a few cases of methaemoglobinaemia were reported with water containing less than 50 mg/litre).

Commoner et al. (1972) found small, but statistically significant, subclinical elevations in methaemoglobin levels in adults exposed to high intakes of nitrates in a rural area when compared with an urban control population. There is also a small amount of data showing that under similar conditions, pregnant women in rural areas have higher methaemoglobin levels than those in urban areas. This is of particular interest since previous workers have reported an increased susceptibility to nitrates in pregnant women. (Skrivan, 1971). Thus, there is concern over the effects on the fetus of the general lowering of oxygen tension. Gelperin et al. (1971) recently reported the presence of methaemoglobinaemia in a newborn infant, presumably exposed to nitrates transplacentally. In this study, 72 mothers and infants tested were exposed to water with nitrate concentrations ranging from 28 to 45 mg/litre over a 2-month period. During the 2 weeks of maximum concentration (45 mg/litre), the average methaemoglobin levels were 1.18% for mothers and 1.91% for newborns with those of one mother and one infant rising to 6.39% and 5.87%, respectively.

Children aged 12–14 years who drank water with a nitrate level of 105 mg/litre were noted to have slightly delayed reactions to light and sound stimuli combined with a mean methaemoglobin level of 5.3% in comparison with control children drinking water with a nitrate level of 8 mg/litre whose methaemoglobin levels average 0.75% (Petukhov & Ivanov, 1970).

8.1.1.2 *Exposure through vegetables*

Cases of methaemoglobinaemia, some resulting in death, have been observed following the consumption of spinach. Hölscher & Natzschka (1964) reported 2 cases in young infants (aged 2 and 3.5 months) who had eaten spinach purée. Fresh spinach from the same source contained only traces of nitrates but had nitrite ion levels of 2180 mg/kg. Fourteen further cases of methaemoglobinaemia in infants (aged 2–10 months) were reported from the Federal Republic of Germany (Sinios & Wodsak, 1965). Unprocessed spinach was used almost exclusively in the preparation of the infants' meals and preparation took place at least 24 h before the mealtime. Since in most cases some of the same spinach had been eaten 24 and 48 h before without causing illness, the authors assumed that the nitrites were formed within the final 24 h of storage. Information on 7 cases showed that the mother tasted the spinach before feeding the child and no change in taste was apparent. None of the children refused the meal which caused the poisoning. Conversion of nitrates to nitrites in fresh spinach was demonstrated by Schuphan (1965) (section 4.2.1). Keating et al. (1973) reported a case of methaemoglobinaemia in an infant given carrot juice. Other cases of food-induced methaemoglobinaemia have recently been reviewed by Luhrs (1973).

8.1.1.3 *High accidental exposures through food*

Certain meat products contain nitrates and nitrites. Normally, no adverse effects result from consumption of such products. However, in 1955, an outbreak of 10 cases of methaemoglobinaemia in children occurred in New Orleans, USA, attributed to the consumption of large amounts of nitrites in sausage meats (Orgeron et al., 1957). Further studies revealed that the meats had nitrite concentrations of more than 200 mg/kg and that some had levels as high as 6570 mg/kg. Singley (1962) reported 3 cases of methaemoglobinaemia resulting from the consumption of fish, that had been adulterated with sodium nitrite. One patient died and it was assessed that he had consumed approximately 33 mg sodium nitrite/kg body weight. Other cases of poisoning involving the consumption of nitrite-treated sausage and frankfurters were reported by Bakshi et al. (1967) and Henderson & Raskin (1972).

8.1.1.4 *Ambient air exposures*

Effects on man of nitrate aerosols in ambient air were not considered by the Task Group. However, because of the recent concern with the role of nitrates in urban air pollution, it may be of interest to briefly summarize the current information on this problem. Airborne nitrates may act as respiratory irritants (Knelson & Lee, 1977) and a recent study

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conducted by the US Environmental Protection Agency in the New York New Jersey metropolitan area showed that increased asthmatic attacks were significantly associated with elevated levels of suspended nitrates in six of the seven communities studied. No such effect was observed with nitrogen dioxide. Another study in two south-eastern communities in the USA showed some evidence that a combination of suspended nitrates and suspended sulfates increased the risk of asthma attacks more than either pollutant did alone. Because of the present difficulties in measuring suspended nitrates, these results should be considered as qualitative instead of quantitative (French et al., unpublished data)[a].

8.1.2 Factors involved in susceptibility to nitrates

The work of Marriott et al. (1933), who investigated the acidity and bacterial flora of 200 infants suffering from diarrhoea, supports the hypothesis that lack of acidity in the gastric juices of newborn infants might permit the growth of nitrate-reducing organisms in the upper gastrointestinal tract and, thus, the reduction of nitrates to nitrites before the former could be completely absorbed. The pH of the stomach contents of healthy infants varied from 2.0 to 5.0 (average 3.7) and that for infants suffering from bacillary dysentery ranged from 2.0 to 5.0 (average 3.0). However, for infants with nonspecified diarrhoea the pH varied from 4.6 to 6.5 (average 5.6).

According to estimates made by Burden (1961), the water intake of young infants is nearly 10 times higher than that of adults on a per unit body weight basis.

The susceptibility of infants to methaemoglobinaemia during the first six months of life, and especially during the first trimester (Bailey, 1966; Kübler, 1965) can be explained by various mechanisms, none of which is fully understood at present. It has been reported that fetal haemoglobin, which in newborn infants makes up to 60–80% of the total haemoglobin decreasing to about 20–30% in 3 months (British Medical Journal, 1966; Kübler, 1965), is more readily oxidized to methaemoglobin than adult haemoglobin (Betke, 1953; Künzer & Schneider, 1953). This might explain why premature infants, who frequently have a higher percentage of fetal haemoglobin than full-term infants are more susceptible to methaemoglobinaemia. Keohane & Metcalfe (1960) reported that the sensitivity of erythrocytes to oxidation to methaemoglobin on exposure to nitrites gradually declined during childhood until the age of puberty, after which it decreased rapidly. This decline in sensitivity was not

[a] French, J. G., Hasselblad, V., & Johnson, R. Aggravation of asthma by air pollutants. 1971–72 Southeastern CHESS studies.

related to the disappearance of fetal haemoglobin and the authors suggested that some other factor might be responsible. The susceptibility of newborn and young infants to develop methaemoglobinaemia could also be attributed to the incomplete development of the NADH–methaemoglobin reductase system. Several studies have shown that the erythrocytes of newborn and young infants have a lower capacity to reduce methaemoglobin than those of older children and adults and that the erythrocytes of premature newborns have a lower reduction capacity than those of full-term infants (Bartos et al., 1966; Ross, 1963; Ross & Desforges, 1959). Except in rare cases of hereditary enzyme deficiency (Balsamo et al., 1964), this deficiency in the NADH–methaemoglobin reductase system seems to disappear after the first 3–4 months of life (Bartos et al., 1966; Künzer & Schneider, 1953).

The haemoglobin of pregnant women and that of patients suffering from carcinomata has also been reported to be sensitive to oxidation to methaemoglobin. However, this sensitivity disappeared in the first instance after delivery and in the second, after radical extirpation of the neoplasms (Metcalf, 1961).

8.1.3   Dose-response relationships for nitrates and nitrites

Estimates of the effective exposure of the general population to nitrates and nitrites have been discussed briefly in section 5.1.4. However, this information is not sufficient to relate environmental concentrations to actual intake of nitrates or nitrites in cases of observed methaemoglobinaemia. Retrospective epidemiological studies discussed in section 8.1.1 provide little information on intake because in most cases either the data concerning concentrations in water or food were not reliable enough, or the amounts of water or food consumed had not been measured. However, a study reported by Winton et al. (1971) considered the nitrate intake from water in some detail. The study was conducted in southern California and central Illinois, USA, and involved 111 infants whose ages ranged from less than two weeks to six months. The mother of each infant was asked about the fluid intake of the infant in the previous 24 h, the method of formula preparation, and the possible inclusion of any other source of nitrate in the diet (e.g. vegetables) or administration of methaemoglobin-forming medicines. The variables that determined the nitrate dose were the daily fluid intake per body weight (which might increase in hot and arid climates, or with fever), the fraction of the total daily fluid intake taken in the form of water, and the concentration of nitrates in the water. Using this method, it was possible to estimate that a daily dose of 10 mg/kg body

weight could be obtained from water containing a nitrate concentration of 50 mg/litre. The daily water intake varied from 10% of the total daily fluid intake for breast-fed infants or for those receiving ready-to-feed preparations to 90% for infants who were fed preparations made with powder. This shows that no generalization is possible, at present, about the relationship between the nitrate concentration in drinking water and the dose of nitrate, and that estimates of nitrate dose from nitrate concentrations in water or food would have to be made for individual cases, taking into account local conditions and dietary habits.

The relationship between the doses of nitrate and methaemoglobin levels is even more difficult to establish because of large individual differences in response, depending on age and many other host variables. For example, using the method described in the previous paragraph, Winton et al. (1971) found that in a group of 111 infants, 63 received a nitrate dose of less than 1 mg/kg body weight, 23 were exposed to 1–4.9 mg/kg, 20 to 5.0–9.9 mg/kg, and 5 infants to 10–15.5 mg/kg. However, only 3 infants appeared to have methaemoglobin levels above normal (0–2.9%) and they were the youngest of the five who had received more than 10 mg/kg. The highest methaemoglobin level (5.3%) was found in a 30-day-old baby who had received 15.5 mg/kg. Another possible approach is to analyse the available retrospective epidemiological data. This has been done by the Committee on Nitrate Accumulation (1972) and by Diskalenko (1968) who concluded that only very crude estimates of correlations between nitrate concentration in water and methaemoglobin levels could be obtained, probably because of the delay between the analyses of water and blood, difficulties in identifying the sources of water consumed, and the heterogeneity of the population samples studied, particularly with respect to age. The analyses performed by the Committee on Nitrate Accumulation (1972) showed, for example, that an increase in the nitrate concentration in water from 0–49 mg/litre to 49–98 mg/litre, increased the methaemoglobin level, on average, from 1.0% to 1.3% in infants aged 0–3 months, but did not affect the methaemoglobin level (0.8%) in infants aged 3–6 months (Simon et al., 1964). Methaemoglobin and haemoglobin levels, measured in 96 infants from 22 localities in Rheinhessen, Federal Republic of Germany, during official maternal counselling, were correlated with the nitrate concentrations of the drinking water in the localities concerned by Würkert (1974, unpublished data)[a]. Nitrate concen-

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trations of 0–5 mg/litre, 31–50 mg/litre, and over 100 mg/litre corresponded to methaemoglobin levels of 1.65%, 2.44%, and 6.59%, respectively. Higher methaemoglobin levels were found in the presence of infections and in infants given tea to drink or vegetable nutrients.

The last question pertains to the relation between the level of methaemoglobin and clinical signs and symptoms of methaemoglobinaemia. Normally, methaemoglobin is present in the blood at a concentration of less than 2% of total haemoglobin.

Subclinical methaemoglobinaemia (less than 10% methaemoglobin) has not been considered to be of direct health significance, but Petukhov & Ivanov (1970) reported behavioural effects at these levels.

Clinical signs of methaemoglobinaemia such as cyanosis become apparent at a methaemoglobin level of about 10%. Hypoxic signs and symptoms may develop at levels exceeding 20% and death may occur at levels of 50% or more.

In conclusion, the available information does not permit the establishment of a quantitative dose-response relationship for human exposure to nitrates in water or food.

8.2   N-nitroso Compounds

Freund (1937) first described acute intoxication by DMN. Barnes & Magee (1954) described 2 cases of industrial intoxication due to DMN in which one individual had hepatic cirrhosis at death; the other survived but, 6 months later, was shown to have a hard liver suspected of being cirrhotic. Watrous (1947) and Wrigley (1948) reported cases of accidental exposure to N-nitroso-methylurethane. Reddening of the conjunctiva and erythema of the face and feet developed quickly, and a respiratory disorder developed later.

No reports are available concerning carcinogenesis in industrial or other workers exposed to N-nitroso compounds, nor have relationships been established, from epidemiological and analytical data, that link cancer in man with exposure to N-nitroso compounds or their possible precursors such as nitrates, nitrites, and compounds that can be nitrosated, occurring as food components, drugs, and pesticides. A recent review of these data is available (Mirvish, 1976).

Some reports have been concerned with the possible etiological role of N-nitroso compounds in nasopharyngeal cancer in south-east Asia (Clifford, 1970; Fong & Chan, 1973a) and oesophageal cancer in South Africa, Iran, and China (Burrell et al., 1966; Coordinating Group for Research on the Etiology of Esophageal Cancer of North China, 1974; Day, 1975; Harmozdian et al., 1975). However, no relationship has been

established; nitrosamines were detected in food from these areas but this was not confirmed by mass spectroscopy (Eisenbrand et al., 1976; Purchase et al., 1975).

The epidemiology of stomach cancer has been discussed from a similar point of view by Correa et al. (1975), Endo et al. (1973), Haenzel & Correa (1975), Hill et al. (1973), Mirvish (1971), and Weisburger & Raineri (1975). It was suggested that nitrosamides might be formed in the stomach from amides occurring in the diet, and might then act locally on this organ. Relationships have been sought between the occurrence of stomach cancer and the nitrate contents of the soil or water in Chile, Colombia, and the United Kingdom (Hawksworth et al., 1974; Hill et al., 1973; Zaldivar & Wetterstrand, 1975) but none was established.

9. EVALUATION OF HEALTH RISKS TO MAN FROM EXPOSURE TO NITRATES, NITRITES, AND N-NITROSO COMPOUNDS

9.1 Nitrates and Nitrites

9.1.1 General considerations

Man is exposed to nitrates and nitrites mainly through water and food. Nitrate concentrations may be particularly high in drinking water derived from dug wells. Nitrates in food may occur naturally or may be added for various technological or even public health reasons (e.g. addition of nitrates and nitrites to certain meat products to protect against botulism). Although intake of very large doses of nitrates can be fatal to man, such intake is not likely to occur through environmental exposure, except in the case of infants and very young children who are high risk groups because of their susceptibility to nitrates and nitrites. Weekly intakes of nitrates by members of the general population are difficult to evaluate but rough estimates are available for England and the USA giving values of about 400–450 mg/week (85–105 mg from water; 210–225 mg from vegetables, and about 110 mg from meat products).

These figures cannot be applied generally and a separate estimation of the nitrate intake from food and water should be made for each case, especially when the subjects are infants or young children (section 8.1.3). Exposure to nitrates can also occur through the inhalation of polluted air.

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The assessment of health risks to man (section 9.1.2) has been based on epidemiological studies and clinical evidence. The animal data discussion in section 7.1, confirm the findings in man that methaemoglobinaemia is the main toxic effect of nitrate and nitrite ingestion. Methaemoglobinaemia is caused by nitrites, the reduction products of nitrates. The reduction usually occurs through microbial action either in the environment or in the body. The health risks from exposure to nitrates is therefore related not only to their concentration in drinking water and food, but also to the presence or absence of conditions conducive to their reduction to nitrites. Young infants constitute the most vulnerable group for the following reasons:

(1) Lower acidity in their stomach allows the growth of certain microbes that contain enzymes capable of reducing nitrates to nitrites;

(2) Fetal haemoglobin, which constitutes a considerable proportion of the haemoglobin of the young infant, and the erythrocytes during childhood may be more susceptible to conversion to methaemoglobin by the action of nitrites;

(3) The enzyme system capable of reducing methaemoglobin to haemoglobin is deficient in the young infant; and

(4) The fluid intake of the young infant is higher than that of the adult in relation to the body weight.

9.1.2 Assessment of health risks

Precise dose-response relationships could not be established by the Task Group because of the existence of various strongly modifying factors and the lack of accurate quantitative data. However, on the basis of the available information, the Task Group reached the following conclusions:

(a) *General population* — The prevailing levels of nitrates and nitrites in water and food do not seem to have any harmful effects in adults and older children, although there are reports of susceptible individuals who have been affected by meat treated with nitrites, and of cases of poisoning resulting from the ingestion of certain foods accidently containing excessive amounts of nitrites (8.1.1.3). Subclinical methaemoglobinaemia may also be found in individuals consuming water containing high levels of nitrates (8.1.3).

(b) *Susceptible group* — Infants less than 6 months old and especially those under 3 months of age are particularly susceptible to methaemoglobinaemia caused by intake of water containing elevated levels of nitrates, especially when they are fed with preparations made from dried milk of low acidity. While a few cases of methaemoglobinaemia

have been reported associated with water nitrate levels of less than 50 mg/litre, most cases occur with nitrate levels of 90 mg/litre or more. Nitrates in water may cause death of the infant, but the lowest level that may be fatal cannot be estimated at present.

The ingestion of vegetables (e.g. spinach, carrots) containing elevated nitrate and/or nitrite levels may also cause methaemo-globinaemia in infants, especially in those aged between 6 months and 1 year. Storage of vegetables, other than in the frozen or canned state, is likely to increase the nitrite level and hence the risk.

(c) *Effects of airborne nitrates* — A few recent studies have indicated that airborne nitrates may act as respiratory irritants but, at present, adequate quantitative data are not available and the Task Group did not consider this exposure in their health risk evaluation.

9.2   N-nitroso Compounds

9.2.1   General considerations

Nitrites (and indirectly nitrates) can react with amines and amides to form nitrosamines and nitrosamides. The precursors of these N-nitroso compounds are widely distributed in various environmental media. Information concerning the presence of N-nitroso compounds *per se* is limited although they have been identified in certain foods such as luncheon meats and in air and water samples. The conditions under which N-nitroso compounds can be formed are outlined in sections 4.2 4.6.

More than 80% of over one hundred N-nitroso compounds tested proved to be carcinogenic in animal experiments giving rise to tumours in many organs and also producing tumours transplacentally. N-nitroso compounds are carcinogenic in a wide range of animal species; most are mutagenic in test systems and some have been shown to be teratogenic to animals.

The possible health hazard from N-nitroso compounds is not confined to those present in the environment. Their formation, from a variety of precursors in the body of animals, has been demonstrated, and this may also occur in man.

9.2.2   Assessment of health risks

A dose-response relationship has been shown to exist in different species of rodents for some carcinogenic N-nitroso compounds. As the dose is reduced, the tumour incidence decreases and the time for tumour induction increases and may exceed the life span of the animals.

Although there is no clinical or epidemiological evidence, it is highly probable that these compounds are also carcinogenic to man. However, present limitations concerning available dose-response data in animals and their interpretation, and inadequate knowledge of the biomechanism of cancer induction preclude a quantitative estimation of the carcinogenic risk to man that may be associated with different exposures to N-nitroso compounds.

9.3 Reduction of Exposure

The assessments of health risk given in sections 9.1.2 [(a) and (b)] and 9.2.2 lead to a number of practical conclusions concerning the need to reduce the exposure to nitrates, nitrites, and N-nitroso compounds.

As regards the exposure to nitrates and nitrites, the Task Group made the following specific recommendations:

(a) Infant dried milk preparations should be reconstituted only with water containing low levels of nitrates. If such water is not available, breast feeding or the use of cow's milk should be encouraged.

(b) Only vegetables with a low nitrate content should be used in the preparation of baby foods. If vegetables known to contain high levels of nitrates are used, appropriate food processing precautions should be instituted. Nitrates and nitrites should not be added to baby foods.

(c) The use of nitrates and nitrites in foods as preservatives should be reduced to the minimum level that provides protection against botulism. This applies particularly to cured and canned meats and to fish. The use of nitrates and nitrites on fresh meats or fish should be avoided.

(d) Nitrate levels in public drinking water should comply with, or preferably be lower than, the tentative limit of 45 mg/litre recommended in the International Standards for Drinking Water (WHO, 1971).

With respect to the carcinogenic risk from exposure to N-nitroso compounds, it is prudent to assume that any exposure may involve some degree of risk, and that exposure should therefore be kept as low as practically achievable. This may not be an easy task in many instances, since these compounds may occur in the environment in concentrations of the order of parts per billion, as a result of a variety of natural and technological processes, and, moreover, they may be formed *in vivo* from nitrates, amines, and amides, which are ubiquitous. Obviously the recommendations (a) to (d) will also contribute to the reduction of carcinogenic risk related to N-nitroso compounds.

REFERENCES

ACHTZEHN, M. K. & HAWAT, H. (1969) [Nitrate accumulation in vegetables a possibility of nitrate intoxication in infants.] *Die Nahrung*, **13**: 667 676 (in German).

ACHTZEHN, M. K. & HAWAT, H. (1970) [On nitrate formation in vegetables and vegetable products. Part I. Raw spinach.] *Die Nahrung*, **14**: 383–394 (in German).

ADRIANO, C. C., PRATT, D. F., & BISHOP, S. E. (1971) Fate of inorganic forms of nitrogen and salt from land disposed manures from dairies. In: *Livestock waste management and pollution abatement*. St. Joseph, MI (Am. Soc. Agric. Eng. Proc. No. 271).

ALAM, B. S., SAPORESCHETZ, I. B., & EPSTEIN, S. S. (1971a) Formation of *N*-nitrosopiperidine from piperidine and sodium nitrite in the stomach and the isolated intestinal loop of the rat. *Nature (Lond.)*, **232**: 116 118.

ALAM, B. S., SAPORESCHETZ, I. B., & EPSTEIN, S. S. (1971b) Synthesis of nitrosopiperidine from nitrate and piperidine in the gastro-intestinal tract of the rat. *Nature (Lond.)*, **232**: 199–200.

ALARIF, A. & EPSTEIN, S. S. (1974) *The uptake and metabolism of* [14]*C-labelled nitrosomethylurethane and nitrosomethylurea in guinea pigs and their* in vitro *metabolism in the guinea pig and human pancreas*. IARC Sci. Publ. No. 9, pp. 215–219.

ALEXANDROV, V. A. (1967) [On the mechanism of carcinogenicity of *N*-nitrosodimethylamine in connection with peculiarities of its effect on embryogenesis] *Vop. Onkol.*, **13**: 87–92 (in Russian).

ALEXANDROV, V. A. (1968a) Blastomogenic effect of dimethylnitrosamine on pregnant rats and their offspring. *Nature (Lond.)*, **218**: 280–281.

ALEXANDROV, V. A. (1968b) [Effect of *N*-nitroso-*N*-methylaniline and *N*-nitroso-*N*-ethylaniline on the rat embryo] *Vop. Onkol.*, **14**: 37–38 (in Russian).

ALEXANDROV, V. A. (1969a) [Transplacental blastomogenic action of *N*-nitrosomethylurea in rat offspring] *Vop. Onkol.*, **15**: 55 61 (in Russian).

ALEXANDROV, V. A. (1969b) Uterine, vaginal and mammary tumors induced by nitrosoureas in pregnant rats. *Nature (Lond.)*, **222**: 1064–1065.

ALEXANDROV, V. A. & JANISCH, W. (1971) Teratogenic effect of ethylurea and nitrite in rats. *Experientia (Basel)*, **27**: 538 539.

ALLISTON, T. G., COX, G. B., & KIRK, R. S. (1972) The determination of steam-volatile *N*-nitrosamines in foodstuffs by formation of electron-capturing derivatives from electrochemically derived amines. *Analyst*, **97**: 915–920.

ALTHOFF, J., WILSON, R., & MOHR, U. (1971) Diethylnitrosamine-induced alterations in the tracheobronchial system of Syrian golden hamsters. *J. Natl Cancer Inst.*, **46**: 1067-1071.

ALTHORPE, J., GODDARD, D. A., & SISSONS, D. J., TELLING, G. M. (1970) The gas chromatographic determination of nitrosamines at the picogram level by conversion to their corresponding nitramines. *J. Chromatogr.*, **53**: 371 -373.

ARENA, J. M. (1970) *Poisoning, toxicology, symptoms and treatment*. 2nd ed., Springfield, I, Bannerstone House, p. 9.

ARISON, R. N. & FEUDALE, E. L. (1967) Induction of renal tumour by streptozotoc n in rats. *Nature (Lond.)*, **214**: 1254–1255.

ASAHINA, S., FRIEDMAN, M. A., ARNOLD, E., MILLAR, G. N., MISHKIN, M., BISHOP, Y., & EPSTEIN, S. S. (1971) Acute synergistic toxicity and hepatic necrosis following oral administration of sodium nitrite and secondary amines to mice. *Cancer Res.*, **31**: 1201–1205.

ASATOOR, A. M. & SIMENHOFF, M. L. (1965) The origin of urinary dimethylamine. *Biochim. Biophys. Acta*, **111**: 384 392.

ASHTON, M. R. (1970) The occurrence of nitrates and nitrites in foods. *BFMIRA lit. surv.* No 7, pp. 32.

BADER, G., STILLER, D., & ULLRICH, K. (1971) [Ultrastructural changes in toxically injured and proliferating liver cells due to long-term application of diethylnitrosamine] *Arch. Geschwulstforsch.*, **37**: 327 343 (in German).

BAILEY, W. P. (1966) Methemoglobinemia– acute nitrate poisoning in infants: Second report. *J. Am. Osteopath. Assoc.*, **66**: 431 434.

BAKSHI, S. P., FAHEY, J. L., & PIERCE, L. E. (1967) Sausage cyanosis—Acquired methemoglobinemia nitrite poisoning. *N. Eng. J. Med.* **277**: **1072.**

BALSAMO, P., HARDY, W. R., & SCOTT, E. M. (1964) Hereditary methaemoglobinaemia due to diaphorase deficiency in Navajo Indians. *J. Pediat.*, **65**: 928–931.

BANNASCH, P. (1968) In: *The cytoplasm of hepatocytes during carcinogenesis.* New York, Springer Verlag.

BARNES, J. M. & MAGEE, P. M. (1954) Some toxic properties of dimethylnitrosamine. *Br. J. ind. Med.*, **11**: 167–174.

BARTOS, H. R., DESFORGES, J. F., & CLARK, N. (1966) Erythrocyte DPNH dependent diaphorase levels in infants. *Pediatrics*, **37**: 991 993.

BEHROOZI, K., GUTTMAN, R., GRUENER, N., & SHUVAL, H. I. (1971) Changes in the motor activity of mice given sodium nitrite drinking solution. In: *Proceedings of a Symposium on Environmental Physiology, Beersheba, December, 1971.*

BNEMANSKIJ, V. V. & LITVINOV, N. N. (1969) [Some data on the histo- and morphogenesis of experimental tumours of the kidneys in animals.] *Arkh. Pat.*, **31**: 79 84 (in Russian).

BERTRAM, J. S. & CRAIG, A. W. (1972) Specific induction of bladder cancer in mice by butyl- (4-hydroxybutyl) -nitrosamine and the effects of hormonal modifications on the sex difference in response. *Eur. J. Cancer,* **8** (6): 587 594.

BERTRAM, J. S. & CRAIG, A. W. (1973) Induction of bladder tumours in mice with dibutylnitrosamine. *Br. J. Cancer,* **24** (2): 352- 359.

BETKE, K. (1953) [Comparative examination of the oxidation of fetal and adult oxyhaemoglobin by sodium nitrite.] *Naturwiss.*, **40**: 60 (in German).

BHATHAL, P. S. & HURLEY, J. V. (1973) An electron microscope study of the production of ascites in acute dimethylnitrosamine (NDMA)-induced liver injury. *J. Pathol.*, **111**: 103–116.

BIOČEV, IV. & POČINKOVA, Z. (1972) [Effect of applied artificial fertilizers on the soil, drinking water and foodstuffs of vegetable origin. *Report on the International Congress of Country Hygiene, Varna*] (in Russian).

BLATTMANN, L. & PREUSSMAN, R. (1973) [The structure of metabolites of carcinogenic dialkylnitrosamine in the urine of rats] *Z. Krebsforsch.*, **79**: 3 5 (in German).

BLATTMANN, L. & PREUSSMAN, R. (1974a). [The biotransformation of carcinogenic dialkylnitrosamines. Further urine metabolites of di-n-butyl- and di-n-pentylnitrosamines.] *Z. Krebsforsch.*, **81**: 75 78 (in German).

BLATTMANN, L., JOSWIG, N., & PREUSSMAN, T. (1974b). [The structure of the metabolites of the carcinogenic methyl-n-butyl-nitrosamine in the urine of rats.] *Z. Krebsforsch.*, **81**: 71–73 (in German).

BODIPHALA, T. & ORMROD, D. P. (1971) Factors affecting the nitrate content of vegetable and fruit foods. *Can. Inst. Food Technol., J.*, **4** (1): 6–8.

BOGOVSKI, P. & WALKER, E. A., ed. (1974) *N*-nitroso compounds in the environment. *Proceedings of a Working Conference, the International Agency for Research on Cancer, Lyons, France, 17 20 October, 1973*, Lyons, International Agency for Research on Cancer, pp. 243.

BOGOVSKI, P., PREUSSMANN, R., & WALKER, E. A., ed. (1972a) *N*-nitroso compounds analysis and formation. *Proceedings of a Working Conference, Deutsches Krebsforschungszentrum, Heidelberg, Federal Republic of Germany, 13 15 October, 1971,* Lyons, International Agency for Research on Cancer, pp. 140.

BOGOVSKI, P., CASTEGNARO, M., PIGNETTELI, B., & WALKER, E. A. (1972b) *The inhibiting effects of tannins on the formation of nitrosamines.* IARC Sci. Publ. No. 3, pp. 127 129.

BOLIN, B. & ARRHENIUS, E., ed. (1977) An essential life factor and a growing environmental hazard. Report from Nobel Symposium No. 38, *Ambio,* **6** (2–3): 96 105.

BOSCH, H. M., ROSENFIELD, A. B., HUSTON, R., SHIPMAN, H. R., & WOODWARD, F. L.

(1950) Methemoglobinemia and Minnesota well supplies. *J. Am. Water Assoc.*, **42**: 161–170.

BOYLAND, E. & WALKER, S. A. (1974) Effect of thiocyanate on nitrosation of amines. *Nature (Lond.)*, **248**: 601–602.

BOYLAND, E., CARTER, R. L., GORROD, J. W., & ROE, F. J. C. (1968) Carcinogenic properties of certain rubber additives. *Eur. J. Cancer*, **4**: 233–239.

BRESLER, S. E., KALININ, V. L., & PERUMOV, D. A. (1968) Inactivation and mutagenesis on isolated DNA. IV Possibility of integration of lethal damage into the chromosome of *Bacillus subtilis* during transformation. *Mutat. Res.*, **5**: 329–341.

BRETSCHNEIDER, K. & MATZ, J. (1973) [Nitrosamines (NA) in the atmospheric air and in the air at the workplace.] *Arch. Geschwulstforsch.*, **42**: 36–41 (in German).

BRETSCHNEIDER, K. & MATZ, J. (1976) *Occurrence and analysis of nitrosamines in air.* IARC Sci. Publ. No. 14, pp. 395–399.

British Medical Journal (1966), **1**: 250–251. Spinach—a risk to babies.

BROWN, J. R. & SMITH, G. E. (1967) *Nitrate accumulation in vegetable crops as influenced by soil fertility practises.* Columbia, University of Missouri, pp. 43 (Res. Bull. No. 920).

BRUNI, C. (1973) Distinctive cells similar to fetal hepatocytes associated with liver carcinogenesis by diethylnitrosamine. Electron microscopic study. *J. Natl Cancer Inst.*, **50**: 1513–1528.

BUGLASS, A. J., CHALLIS, B. C., & OSBORNE, M. R. (1974) *Transnitrosation and decomposition of N-nitrosamines. N-nitroso compounds in the environment.* IARC Sci. Publ., No. 9, pp. 94–100.

BURDEN, E. H. W. J. (1961) The toxicology of nitrates and nitrites with particular reference to the potability of water supplies. *Analyst*, **86** (1024): 429–433.

BURNS, R. C. & HARDY, R. W. F. (1975) *Nitrogen fixation in bacteria and higher plants.* Berlin, Springer-Verlag.

BURRELL, R. J. W., ROACH, W. A., & SHADWELL, A. (1966) Esophageal cancer in the Bantu of the Transkei associated with mineral deficiency in garden plants. *J. Natl Cancer Inst.*, **36**: 201–214.

BUTLER, W. H. & HARD, G. C. (1971) Hepatoxicity of dimethylnitrosamine in the rat with special reference to veno-occlusive disease. *Exp. mol. Pathol.*, **15**: 209–219.

CALAFAT, J., DEN ENGELSE, L., & EMMELOT, P. (1970) Studies on lung tumours. II. Morphological alterations induced by dimethylnitrosamine in mouse lung and liver and their relevance to tumourigenesis. *Chem. biol. Interact.*, **2**: 309–320.

CARDESA, A., MIRVISH, S. S., HAVEN, G. T., & SHUBIK, P. (1974) Inhibitory effect of ascorbic acid on the acute toxicity of dimethylamine plus nitrite in the rat. *Proc. Soc. Exp. Biol. Med.*, **145**: 124–128.

CARTER, R. L., PERCIVAL, W. H., & ROE, F. J. C. (1969) Exceptional sensitivity of mink to the hepatotoxic effects of dimethylnitrosamine. *J. Pathol.*, **97** (1): 79–88.

CASEY, H. (1975) Variation in chemical composition of the River Frome, England from 1965 to 1972. *Freshwater Biol.*, **5**: 507–514.

CASTEGNARO, M., PIGNATELLI, B., & WALKER, E. A. (1972) N-*Nitroso compounds: Analysis and formation.* IARC Sci. Publ. No. 3, pp. 87–89.

CAVETT, J. J. (1962) The microbiology of vacuum-packed sliced bacon. *J. appl. Bact.*, **25**: 282–289.

CHALLIS, B. C. (1973) Rapid nitrosation of phenols and its implications for health hazards from dietary nitrites. *Nature (Lond.)*, **244**: 466.

CHALLIS, B. C. & BARTLETT, C. D. (1975) Possible cocarcinogenic effects of coffee constituents. *Nature (Lond.)*, **254**: 532–533.

CHALLIS, B. C. & CHALLIS, J. A. (1970) Reactions of the carboxamide group. In: Zabicky, J., ed. *The chemistry of amides*, London, Interscience, pp. 733–848.

CIAVAGLIA, F. & THOMPSON, R. P. (1969) Infant methemoglobinemia. Absence in areas with low concentrations of nitrate–nitrogen in water supply. *N.Y. State J. Med.*, **69**: 3128–3129.

CLAPP, N. K. & TOYA, R. E. (1970) Effect of cumulative dose and dose rate on dimethylnitrosamine oncogenesis in RF mice. *J. Natl Cancer Inst.*, **45**: 495–498.

CLAPP, N. K., TYNDALL, R. L., & OTTEN, J. A. (1971) Differences in tumor types and organ susceptibility in BALB-C and RF mice following dimethylnitrosamine and diethylnitrosamine. *Cancer Res.*, **31**: 196–198.

CLIFFORD, P. (1970) On the epidemiology of nasopharyngeal carcinoma. *Int. J. Cancer*, **5**: 287–309.

COLLINS-THOMPSON, D. L., SEN, N. P., ARIS, B., & SCHWENGHAMER, L. (1972) Non-enzymic *in vitro* formation of nitrosamines by bacteria isolated from meat products. *Can. J. Microbiol.*, **18** (12): 1968–1971.

COMLY, H. H. (1945) Cyanosis in infants caused by nitrates in well water. *J. Am. Med. Assoc.*, **129**: 112–116.

COMMITTEE ON NITRATE ACCUMULATION (1972) *Accumulation of nitrate*. Washington, DC, National Academy of Sciences. p. 48.

COMMONER, B. (1970) Threats to the integrity of the nitrogen cycle. In: Singer, F. S., ed. *Global effects of environmental pollution*, NY, Springer Verlag, pp. 70–95.

COMMONER, B., SHEARER, G., & KOHL, D. (1972) *A study of certain ecological, public and economic consequences of the use of inorganic nitrogen fertilizer*, St. Louis, Washington University. (1st year Progress Report NSF Grant No. GI29926x).

COOKE, G. E. & WILTIAMS, R. J. B. (1970) Losses of nitrogen and phosphorus from agricultural land. *Water Treat. Exam.*, **19** (3): 253–276.

COORDINATING GROUP FOR RESEARCH ON THE ETIOLOGY OF ESOPHAGEAL CANCER OF NORTH CHINA (1974) *The epidemiology of esophageal cancer in North China and preliminary results in the investigation of its etiological factors*. Abstract, *11th International Cancer Congress, Florence, Italy*.

CORREA, P., HAENSZEL, W., CUELLO, C., TANNENBAUM, S., & ARCHER, M. (1975) A model for gastric cancer epidemiology, *Lancet*, July 12, pp. 58–59.

COUCH, D. B. & FRIEDMAN, M. A. (1975) Interactive mutagenicity of sodium nitrite, dimethylamine, methylurea and ethylurea, *Mutat. Res.*, **31**: 109–114.

CRADDOCK, V. M. (1971) Liver carcinomas induced in rats by single administration of dimethylnitrosamine after partial hepatectomy. *J. Natl Cancer IM NST.*, **47**: 899–907.

CROSBY, N. T., FOREMAN, K. K., PALFRAMEN, J. F., & SAWYER, R. (1972) *Determination of volatile nitrosamines in food products at the µg/kg level*. IARC Sci. Publ. No. 3, pp. 38–42.

DAIBER, D. (1966) [*Methods for the quantitative and qualitative estimation of organic N-nitroso compounds*.] Freiburg (Thesis) (in German).

DAY, N. E. (1975) Some aspects of the epidemiology of esophageal cancer. *Cancer Res.*, **35** (2): 3304–3307.

DELWICHE, C. C. (1970) The nitrogen cycle. *Sci. Am.*, **223**: 137–146.

DISKALENKO, A. P. (1968) Methemoglobinemia of water–nitrate origin in the Moldavian SSR. *Gig. i Sanit.*, **33**: 32–37.

DISKALENKO, A. P. (1969) [*Methaemoglobinaemia caused by nitrates in water and its prophylaxis.*] Kišinev (in Russian).

DISKALENKO, A. P. & DOBRJANSKAJA, E. V. (1972) [Changes in redox processes in hepatic and cerebral tissues as a result of the ingestion of nitrates with drinking water. In: *Topical questions of hygiene and epidemiology*] Kišinev, pp. 22–23 (in Russian).

DISKALENKO, A. P. & TROFIMENKO, JU. N. (1972) [Changes in the activity of the glutathione-ascorbic acid system and respiration in erythrocytes as a result of the ingestion of nitrites with drinking water. In: *Topical questions of hygiene and epidemiology* Kišinev, pp. 23–25 (in Russian).

DOWNS, E. F. (1950) Cyanosis of infants caused by nitrate concentrations in rural water-supplies. *Bull. World Health Organ.*, **3**: 165–169.

DRUCKREY, H. & PREUSSMANN, R. (1962) [The production of lung cancer in rats by subcutaneous injection of diamylnitrosamine.] *Naturwiss.*, **49**: 111–112 (in German).

DRUCKREY, H., PREUSSMAN, R., SCHMÄHL, D., & MÜLLER, M. (1961) [The chemical constitution and carcinogenic effects of the nitrosamines.] *Naturwiss.*, **48**: 134–135 (in German).

DRUCKREY, H., STEINHOFF, D., BEUTHNER, H., SCHNEIDER, H., & KLÄRNER, P. (1963a)

88

[Screening of nitrite for chronic toxicity in rats.] *Arzneimittel-Forsch.*, **13**: 320–323 (in German).

DRUCKREY, H., SCHILDBACH, A., SCHMÄHL, D., PREUSSMANN, R., & IVANKOVIC, S. (1963b) [Quantitative analysis of the carcinogenic action of diethylnitrosamine.] *Arzneimittel-Forsch.*, **13**: 841–851 (in German).

DRUCKREY, H., IVANKOVIC, S., & PREUSSMANN, R. (1966) Teratogenic and carcinogenic effects in the offspring after single injection of ethylnitrosourea in pregnant rats. *Nature (Lond.)*, **210**: 1378–1379.

DRUCKREY, H., PREUSSMANN, R., IVANKOVIC, S., & SCHMÄHL, D. (1967) [Organotropic carcinogenic effects of 65 different *N*-nitroso-compounds in BD-rats.] *Z. Krebsforsch.*, **69**: 103–201 (in German).

DRUCKREY, H., LANDSCHÜTZ, CH., & PREUSSMANN, R. (1968) [Oesophageal carcinoma due to inhalation of methylbutylnitrosamine (MBNA) in rats.] *Z. Krebsforsch.*, **71**: 135–139 (in German).

DRUCKREY, H., LANDSCHÜTZ, CH., & IVANKOVIC, S. (1970a) [Transplacental production of malignant tumours in the nervous system II. Ethylnitroso urea in 10 genetically defined rat strains.] *Z. Krebsforsch.*, **73**: 371–386 (in German).

DUBROW, H. & KABISCH, W. (1960) [Investigations of the evidence of nitrates and nitrites in cheese.] *Milchwiss.*, **15** (11): 543–549 (in German).

DURFOR, C. N. & BECKER, E. (1965) *Public water supplies of the 100 largest cities in the United States.* Washington DC, US Dept Interior (Geological Survey Water Supply Paper 1812).

DUTTON, A. H. & HEATH, D. F. (1956) Demethylation of dimethylnitrosamine in rats and mice. *Nature (Lond.)*, **178**: 644.

EISENBRAND, G. (1972) *Determination of volatile nitrosamines at low levels in food by acid-catalysed denitrosation and formation of derivatives from the resulting amines.* IARC Sci. Publ. No. 3, pp. 64–71.

EISENBRAND, G. (1973) *Determination of volatile nitrosamines: a review.* In: *Proceedings of the International Symposium on Nitrite in Meat Products.* Zeist, pp. 45–52.

EISENBRAND, G. & PREUSSMANN, R. (1970) [A new method for the colorimetric determination of nitrosamines following cleavage of the *N*-nitroso group with hydrogen bromide in glacial acetic acid.] *Arzneimittel-Forsch.*, **20**: 1513–1517 (in German).

EISENBRAND, G., JANZOWSKI, C., & PREUSSMANN, R. (1975) Gas chromatographic determination of *N*-nitrosoamino acids by trimethylsilylation and single-ion mass fragmentography. *J. Chromatogr.*, **115**: 602–606.

EISENBRAND, G., VON RAPPARD, E., ZAPPE, R., & PREUSSMANN, R. (1976) *Trace analysis of volatile nitrosamines by a modified nitrogen specific detector in pyrolytic mode and by ion-specific determination of heptafluorobutyramides in a GC/MS system.* IARC Sci. Publ. No. 14, pp. 65–75.

EISENSTARK, A. & ROSNER, J. L. (1964) Chemically induced reversions in the *cysC* region of *Salmonella typhimurium. Genetics,* **49**: 343–355.

ELESPURU, R. K. & LIJINSKY, W. (1973) The formation of carcinogenic nitroso compounds from nitrite and some types of agricultural chemicals. *Food Cosmet. Toxicol.,* **11** (3): 807–811.

ELESPURU, R., LIJINSKY, W., & SETLOW, J. K. (1974) Nitrosocarbaryl as a potent mutagen of environmental significance. *Nature (Lond.),* **247**: 386–387.

ENDER, F. & ČEH, L. (1967) Occurrence and determination of nitrosamines in foodstuffs for human and animal nutrition. *Alkylierend Wirkend Verbindungen, Second Conference on Tobacco Research.,* Freiburg, 1960, pp. 83–91.

ENDER, F. & ČEH, L. Z. (1971) Conditions and chemical reaction mechanisms by which nitrosamines may be formed in biological products with reference to their possible occurrence in food products. *Z. Lebensm. Unters. Forsch.,* **145** (3): 133–142.

ENDER, F., HAVRE, G., HELGEBOSTAD, A., KOPPANG, N., MADSEN, R., & ČEH, L. (1964) Isolation and identification of a hepatoxic factor in herring meal produced from sodium nitrite preserved herring. *Naturwiss.,* **51**: 637–638.

ENDER, F., HAVRE, G., MADSEN, R., ČEH, L., & HELGEBOSTAD, A. (1967) [Studies on conditions under which *N*-nitrosodime-thylamine is formed in herring meal produced from nitrite-preserved herring.] *Z. Tierphysiol. Tierernähr. Futtermittelk.*, **22**: 181–189 (in German).

ENDO, H., TAKAHESHI, K., & AOYAGI, H. (1973) Screening of compounds structurally and functionally related to *N*-methyl-*N*-nitroso guanidine, a gastric carcinogen. *Gann*, **65**: 45 54.

EPPSON, H. F., GLENN, M. W., ELLIS, W. W., & GILBERT, C. S. (1960) Nitrate in the diet of pregnant ewes. *JAVMA*, **137** (10): 611–614.

EPSTEIN, S. (1972) In vivo *studies on interactions between secondary amines and nitrites or nitrates.* IARC Sci. Publ. No. 3, pp. 109–115.

FAN, T-Y. & TANNENBAUM, S. R. (1972) Automatic colorimetric determination of *N*-nitroso compounds. *J. agric. Food Chem.*, **19**: 1267–1269.

FAN, T-Y. & TANNENBAUM, S. R. (1973) Factors influencing the rate of formation of nitrosomorpholine from morpholine and nitrite: acceleration by thiocyanate and other anions. *J. agric. Food Chem.*, **21**: 237–240.

FAO/IAEA Panel of Experts (1974) *Effects of agricultural production on nitrates in food and water with particular reference to isotope studies. Proceedings and Report of a Panel of Experts, Vienna, 4–8 June 1973,* Vienna, International Atomic Energy Agency, pp. 158.

FASSETT, D. W. (1966) Nitrates and nitrites. In: *Toxicants occurring naturally in foods.* Washington, DC, National Academy of Sciences, pp. 250–449 (Publ. No. 1354).

FAZIO, T., DAMICO, J. N., HOWARD, J. W., WHITE, R. H., & WATTS, J. O. (1971a) Gas chromatographic determination and mass spectrometric confirmation of *N*-nitro-sodimethylamine in smoke-processed marine fish. *J. agric. Food Chem.*, **19** (2): 250–253.

FAZIO, T., WHITE, R. H., & HOWARD, J. W. (1971b) Analysis of nitrite- and/or nitrate-processed meats for *N*-nitrosodimethylamine. *J. Assoc. Off. Anal. Chem.*, **54** (5): 1157–1159.

FAZIO, T., HOWARD, J. W., & WHITE, R. (1972) *Multidetection method for analysis of volatile nitrosamines in foods.* IARC Sci. Publ. No. 3, pp. 16–24.

FAZIO, T., WHITE, R. H., DUSOLD, L. R., & HOWARD, J. W. (1973) Nitrosopyrrolidine in cooked bacon. *J. Assoc. Off. Anal. Chem.*, **56** (4): 919–921.

FERGUSON, J. H., MYSLIWY, T. J., & ARCHER, M. C. (1974) *The nitrosation of spermidine and spermine.* IARC Sci. Publ. No. 9, pp. 90 93.

FERON, V. J., EMMELOT, P., & VOSSENAAR, T. (1972) Lower respiratory tract tumors in Syrian golden hamsters after intratracheal instillations of diethylnitrosamine alone and with ferric oxide. *Eur. J. Cancer*, **8**: 445–449.

FIDDLER, W. (1975) The occurrence and determination of *N*-nitroso compounds. *Toxicol. appl. Pharmacol.*, **31**: 352–360.

FIDDLER, W., DOERR, R. C., ERTEL, J. R., & WASSERMAN, A. E. (1971) Gas-liquid chromatographic determination of *N*-nitrosodimethylamine in ham. *J. Assoc. Off. Anal. Chem.*, **54** (5): 1160 1163.

FIDDLER, W., PENSABENE, J. W., DOERR, R. C., & WASSERMAN, A. E. (1972) Formation of *N*-nitrosodimethylamine from naturally occurring quaternary ammonium compounds and tertiary amines. *Nature (Lond.)*, **236**: 307.

FIDDLER, W., PENSABENE, J. W., PIOTROWSKI, E. G., DOERR, R. C., & WASSERMAN, A. E. (1973) Use of sodium ascorbate or erythorbate to inhibit formation of *N*-Nitroso-dimethylamine in frankfurters. *J. Food Sci.*, **38**: 1084.

FIDDLER, W., PENSABENE, J. W., FAGAN, J. C., THORN, E. J., PIOTROWSKA, E. G., & WASSERMAN, A. (1974) The role of lean and adipose tissue on the formation of nitrosopyrrolidine in fried bacon. *J. Food Sci.*, **39**: 1070 1071.

FIESER, L. F. & FIESER, M. (1967) *Reagents for organic synthesis.* New York, Wiley, pp. 747 749.

FINE, D. H. & RUFFEH, F. (1974) *Description of the thermal energy analyser for N-nitroso compounds.* IARC Sci. Publ. No. 9, pp. 40 44.

90

FINE, D. H., RUFEH, F., & LIEB, D. (1974) Group analysis of volatile and non-volatile N-nitroso compounds. *Nature (Lond.)*, **247**: 309–310.

FINE, D. H., HUFFMAN, F., ROUNBEHLER, D. P., & BETCHER, N. M. (1976a) *Analysis of N-nitroso compounds by combined high-performance liquid chromatography and thermal energy analysis.* IARC Sci. Publ. No. 14, pp. 43–50.

FINE, D. H., ROUNBEHLER, D. P., BELCHER, N. M., & EPSTEIN, S. S. (1976b) N-*nitroso compounds in air and water.* IARC Sci. Publ. No. 14, pp. 401–408.

FLAKS, A., HAMLTON, J. M., CLAYSON, D. B., & BURCH, P. R. J. (1973) The combined effect of radiation and chemical carcinogens in female A x 1F mice. *Br. J. cancer*, **28**: 227–231.

FONG, Y. Y. & CHAN, W. C. (1973a) Dimethylnitrosamine in Chinese marine salt fish. *Food Cosmet. Toxicol.*, **11**: 841–845.

FONG, Y. Y. & CHAN, W. C. (1973b) Bacterial production of dimethylnitrosamine in salted fish. *Nature (Lond.)*, **243**: 421–422.

FONG, Y. Y. & WALSH, E. O'F. (1971) Carcinogenic nitrosamines in Cantonese salt-dried fish. *Lancet*, **2**: 1032.

FOOD STANDARDS COMMITTEE (1959) *Preservatives in food.* London, Her Majesty's Stationery Office.

FOSTER, S. S. D. & CREASE, R. I. (1974) Nitrate pollution of chalk groundwater in east Yorkshire — a hydrological appraisal. *J. Inst. Water Eng.*, **28**: 178–194.

FREIMURTH, U. & GLÄSER, E. (1970) [The appearance of nitrosamines in food.] *Die Nahrung.*, **14** (5): 357–361 (in German).

FREUND, H. A. (1937) Clinical manifestations and studies in parenchymatous hepatitis. *Ann. int. Med.*, **10**: 1144–1155.

FRIDMAN, A. L., MUKHAMETŠIN, F. M., & NOMIKOV, S. S. (1971) [Advances in the chemistry of aliphatic nitrosamines.] *Russ. chem. Rev.*, **40** (1): 34–50 (in Russian).

FRIEDMAN, M. A., GREENE, E. J., & EPSTEIN, S. S. (1972) Rapid gastric absorption of sodium nitrite in mice. *J. pharm. Sci.*, **61** (9): 1492–1494.

GARBIDGE, M. G. & LEGATOR, M. S. (1969) A host-mediated microbic assay for the detection of mutagenic contents. *Proc. Soc. Exp. Biol. Med.*, **130**: 831–834.

GARBUZ, A. M. (1968) [*The effect of symptomless methaemoglobinaemia on some bodily functions.*] Author's summary of a thesis for the degree of Candidate of Medical Sciences, Leningrad (in Russian).

GARBUZ, A. M. (1971) [Changes in the body's functional condition in symptomless methaemoglobinaemia. In: *Methaemoglobinaemia caused by various factors and methods of preventing it.*] Summaries of papers read at the First Scientific Conference on Methaemoglobinaemia, Leningrad, pp. 18–21 (in Russian).

GEIL, J. H., STENGER, R. J., BEHKI, R. M., & MORGAN, W. S. (1968) Hepatotoxic and carcinogenic effects of dimethylnitrosamine in low dosage. Light and electron microscopic study. *J. Natl Cancer Inst.*, **40**: 713–730.

GELPERIN, A., JACOBS, E. E., & KLETKE, L. S. (1971) The development of methemoglobin in mothers and newborn infants from nitrate in water supplies. *Ill. med. J.*, **140**: 42–44.

GERRITSEN, G. A. & DE WILLIGEN, A. H. A. (1969) [The nitrite content of potato and corn starch.] *Die Stärke*, **21**: 101–105 (in French).

GILBERTSON, C. B., McCALLA, T. M., ELLIS, J. R., CROSS, O. E., & WOODS, W. R. (1970) *The effect of animal density and surface slope on characteristics of runoff, solid waste, and nitrate movement on unpaved feedlots.* Lincoln, Univ. Nebraska Coll. Agric. and Home Econ.

GOBBI, A., SOVERINI, R., & GRISLER, R. (1974) [Normal methemoglobinemia values observed in 500 inhabitants of the City of Milan.] *Med. Lavoro*, **65**: 306–310 (in Italian).

GOLDSMITH, J. R., ROKAW, S. N., & SHEARER, L. N. (1975) Distributions of percentage methemoglobin in several population groups in California. *Int. J. Epidem.*, **4**: 207–212.

GOTO, M. (1973) Inorganic chemicals in the environment -- with special reference to the pollution problems in Japan. *Environ. Qual. Saf.*, **2**: 72–77.

91

GROENEN, P. J., JONK, C., VAN INGEN, C., & TEN NOEVER DE BRAUW, M. C. (1976) *Determination of eight volatile nitrosamines in thirty cured meat products with capillary gas chromatography-high resolution mass spectrometry: the presence of nitrosodiethylamine and the absence of nitrosopyrrolidine.* IARC Sci. Publ., No. 14, pp. 321–331.

GREENBERG, R. A. (1974) Ascorbate and nitrosamine formation in cured meats. In: *Proceedings of the International Symposium on Nitrite in Meat Products, Zeist, 10–14 September, 1973,* Wageningen, Centre for Agricultural Publishing and Documentation, pp. 179–188.

GREENBLATT, M. (1973) Ascorbic acid blocking of aminopyrine nitrosation in NZO-Bl mice. *J. Natl Cancer Inst.,* **50**: 1055–1056.

GREENBLATT, M. & MIRVISH, S. S. (1973) Dose-response studies with concurrent administration of piperazine and sodium nitrite to strain A mice. *J. Natl Cancer Inst.,* **50** (1): 119–124.

GREENBLATT, M. & RIJHSINGHANI, K. (1969) Comparative cytopathologic alterations induced by alkylnitrosamines in nasal epithelium of the Syrian hamster. *J. Natl Cancer Inst.,* **42**: 421–433.

GREENBLATT, M., MIRVISH, S., & SO, B. T. (1971) Nitrosamine studies: Induction of lung adenomas by concurrent administration of sodium nitrite and secondary amines in Swiss mice. *J. Natl Cancer Inst.,* **46**: 1029–1034.

GREENBLATT, M., KOMMINENI, V., CONRAD, E., WALLCAVE, L., & LIJINSKY, W. (1972) *In vivo* conversion of phenmetrazine into its *N*-nitroso derivative. *Nature (Lond.),* **236** (62): 25–26.

GREENBLATT, M., KOMMINENI, V. R. C., & LIJINSKY, W. (1973) Null effect of concurrent feeding of sodium nitrite and amino acids to MRC rats. *J. Natl Cancer Inst.,* **50**: 799–802.

GRUENER, N. & SHUVAL, H. I. (1970) Health aspects of nitrates in drinking water. In: Shuval, H. I., ed. *Developments in water quality research.* Ann Arbor, Humphrey Science Publ., pp. 89–106.

GRUNTHAL, D., HELLENBROICH, D. O., SÄNGER, P., & MAAS, H. (1970) [The effect of partial hepatectomies on the hepatoma rate after administration of diethyl-nitrosamine.] *Z. Naturforsch.,* **25**: 1277–1281 (in German).

HADJIOLOV, D. (1971) [The inhibition of dimethylnitrosamine carcinogenesis in rat liver by aminoacetonitrile.] *Z. Krebsforsch.,* **76**: 91–92 (in German).

HAENSZEL, W. & CORREA, P. (1975) — Developments in the epidemiology of stomach cancer over the past decade. *Cancer Res.,* **35**: 3452–3459.

HANSEN, H. H. & MUGGIA, F. M. (1971) Treatment of malignant brain tumours with nitrosoureas. *Cancer Chemother. Rep. Part 1,* **55** (2): 99–100.

HARD, G. C. & BUTLER, W. H. (1970a) Cellular analysis of renal neoplasia. Induction of renal tumors in dietary-conditioned rats by dimethylnitrosamine, with a reappraisal of morphological characteristics. *Cancer Res.,* **30**: 2796–2805.

HARD, G. C. & BUTLER, W. H. (1970b) Cellular analysis of renal neoplasia. Light microscope study of the development of interstitial lesions induced in the rat kidney by a single carcinogenic dose of dimethylnitrosamine. *Cancer Res.,* **30**: 2806–2815.

HARD, G. C. & BUTLER, W. H. (1970c) Toxicity of dimethylnitrosamine for the rat testis. *J. Pathol.,* **102**: 201–207.

HARD, G. C. & BUTLER, W. H. (1971a) Ultrastructural aspects of renal adenocarcinoma induced in the rat by dimethylnitrosamine. *Cancer Res.,* **31**: 366–372.

HARD, G. C. & BUTLER, W. H. (1971b) Ultrastructural study of the development of interstitial lesions leading to mesenchymal neoplasia induced in the rat renal cortex by dimethylnitrosamine. *Cancer Res.,* **31**: 337–347.

HARD, G. C. & BUTLER, W. H. (1971c) Morphogenesis of epithelial neoplasms induced in the rat kidney by dimethylnitrosamine. *Cancer Res.,* **31**: 1496–1505.

HARD, G. C., BORLAND, R., & BUTLER, W. H. (1971) Altered morphology and behavior of kidney fibroblasts *in-vitro* following *in-vivo* treatment of rats with a carcinogenic dose of dimethylnitrosamine. *Experientia (Basel),* **27**: 1208–1209.

92

HARMOZDIAN, H., DAY, N. E., ARAMESH, B., & MAHBOUBI, F. (1975) Dietary factors and esophageal cancer in the Caspian littoral of Iran. *Cancer Res.*, **35**: 3493 3498.

HASHIMOTO, Y., SUZUKI, E., & OKADA, M. (1972) [Induction of urinary bladder tumors in ACI/N rats by butyl (3-carboxypropyl) nitrosamine, a major urinary metabolite of butyl- (4-hydroxbutyl) nitrosamine.] *Gann*, **63**: 637–638 (in Japanese).

HAWKSWORTH, G. & HILL, M. J. (1971a) The formation of nitrosamines by human intestinal bacteria. *Biochem. J.*, **122**: 28p–29p.

HAWKSWORTH, G. & HILL, M. J. (1971b) Bacteria and the *N*-nitrosation of secondary amines. *Br. J. Cancer*, **25**: 520–526.

HAWKSWORTH, G., HILL, M. J., GORDILL, G., & CUELLO, C. (1974) *Possible relationship between nitrates, nitrosamines and gastric cancer in S.U. Columbia.* IARC Sci. Publ. No. 9, pp. 229–234.

HEATH, D. F. (1962) The decomposition and toxicity of dialkylnitrosamines in rats. *Biochem. J.*, **85**: 72–91.

HEDLER, L. & MARQUARDT, P. (1968) Occurrence of diethynitrosamine in some samples of food. *Food Cosmet. Toxicol.*, **6**: 341–348.

HEDLIN, R. A. (1971) Nitrate contamination of ground water in the Neepawa-Langruth area of Manitoba. *Can. J. Soil Sci.*, **51**: 75–84.

HEIN, G. E. (1963) Reaction of tertiary amines with nitrous acid, *J. chem. Educ.*, **40** (4): 181–184.

HENDERSON, W. R. & RASKIN, N. H. (1972) "Hot-Dog" headache: Individual suscepti- bility to nitrite. *Lancet*, **2**: 1162–1163.

HEPPEL, L. A. & PORTERFIELD, V. T. (1949) Metabolism of inorganic nitrite and nitrate esters I. The coupled oxidation of nitrite by peroxide-forming systems and catalase. *J. biol. Chem.*, No. 178, pp. 549–556.

HERMANN, H. (1960) [P. Methylnitrosaminebenzaldehyde, a metabolic product of *Clyto- cybe suaveolens*.] *Naturwiss.*, **47**: 162 (in German).

HEYNS, K. (1973) [On nitrosamines in nutrients.] *Getreide Mehl Brot*, **27**: 249–253 (in German).

HICKS, R. M., WAKEFIELD, J. ST. J., & CHOWANIEC, J. (1973) Co-carcinogenic action of saccharin in the chemical induction of bladder cancer. *Nature (Lond.)*, **243**: 347–349.

HILDRUM, K. I., SCANLAN, R. A., & LIBBEY, L. M. (1975) Identification of γ-butenyl-(β- propenyl) nitrosamine, the principal volatile nitrosamine formed in the nitrosation of spermidine or spermine. *J. agric. Food Chem.*, **23** (1): 34–37.

HILL, M. J., HAWKSWORTH, G., & TATTERSALL, G. (1973) Bacteria, nitrosamines and cancer of the stomach. *Br. J. Cancer*, **28** (6): 562 567.

HMSO (1973) *The determination of nitrate-nitrogen in herbage.* London, Her Majesty's Stationery Office, pp. 3 (Tech. Bull. 27).

HMSO (1974) *Annual Report 1972*, London, Her Majesty's Stationery Office, pp. 206.

HOCH-LIGETI, C., ARGUS, M. F., & ARCOS, J. C. (1968) Combined carcinogenic effects of dimethylnitrosamine and 3-methylcholanthrene in the rat. *J. Natl Cancer Inst.*, **40**: 535 549.

HOFFMAN, D., HECHT, S. S., ORNAF, R. M., & WYDNER, E. L. (1974) *N*-nitrosonornicotine in tobacco. *Science*, **186**: 265–267.

HÖLSCHER, P. M. & NATZSCHKA, J. (1964) [Methaemoglobinaemia in young children due to the nitrite contents of spinach.] *Dtsch Med. Wschr.*, **89**: 1751–1754 (in German).

HORN, E. E. & HERRIOT, R. M. (1962) The mutagenic action of nitrous acid on "single- stranded" (denatured) haemophilus transforming DNA. *Proc. Natl Acad. Sci. US.* **48**: 1409 1416.

HORWITZ, W. (1975) *Association of Official Analytical Chemists, Official methods of analysis.* 12th ed. Washington, DC, pp. 1094.

HOWARD, J. W., FAZIO, T., & WATTS, J. O. (1970) Extraction and gas chromatographic determination of *N*-nitrosodimethylamine in smoked fish: application to smoked nitrite-treated chub. *J. Assoc. Off. Anal. Chem.*, **53** (2): 269–274.

INGRAM, M. (1974) The microbiological effects of nitrite. In: *Proceedings of the*

International Symposium on Nitrite in Meat Products, Zeist, 10–14 September, 1973, Wageningen, Centre for Agricultural Publishing and Documentation, pp. 63 75.

IRETON, H. J., MCGIVEN, A. R., & DAVIES, D. J. (1972) Renal mesenchymal tumours induced in rats by dimethylnitrosamine. Light and electron-microscope studies. *J. Pathol.*, **108**: 181–185.

ISSENBERG, P. & TANNENBAUM, S. (1972) *Approaches to the determination of volatile and non volatile N-nitroso compounds in foods and beverages.* IARC Sci. Publ., No. 3, pp. 31 37.

ITO, N., HIASA, Y., TAMAI, A., OKAJIMA, E., & KITAMORA, H. (1969) Histogenesis of urinary bladder tumors induced by *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine in rats. *Gann*, **60**: 401–410.

ITO, N., MAKIURA, S., YOKOTA, Y., KAMAMOTO, Y., HIASA, Y., & SUGIHARA, S. (1971) Effect of unilateral ureter ligation on development of tumors in the urinary system of rats treated with *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine. *Gann*, **62**: 359 365.

IVANKOVIĆ, S. & DRUCKREY, H. (1968) [Transplacental induction of malignant tumours of the nervous system. 1 Ethylnitrosourea (ENU) in BD-1X-rats.] *Z. Krebsforsch.*, **71**: 320 360 (in German).

IVANKOVIĆ, S. & PREUSSMANN, R. (1970) [Transplacental induction of malignant tumours by the oral administration of ethylurea and nitrite in rats.] *Naturwiss.*, **57**: 460 461 (in German).

IVANKOVIĆ, S., PREUSSMANN, R., SCHMÄHL, D., & ZELLER, J. (1973) [Prevention of nitrosamide induced hydrocephali by ascorbic acid after prenatal administration of ethylurea and nitrite to rats.] *Z. Krebsforsch.*, **79**: 145–147 (in German).

JACKSON, W. A., STEEL, J. S., & BOSWELL, V. R. (1967) Nitrates in edible vegetables and vegetable products. *Proc. Am. Soc. Hort. Sci.*, **90**: 349 352.

JAFFÉ, E. R. & HELLER, P. (1964) Methemoglobinemia in man. In: Moore, C. V. & Brown, E. B., ed. *Progress in hematology*, vol. IV, New York, Grune & Stratton, pp. 48 71.

JAPAN ENVIRONMENTAL SANITATION CENTER (1974) [*Report of 1973 results of the analysis for suspended particulate matter in the National Air Sampling Network.*] Kawasaki, Japan, pp. 14, 24 (in Japanese).

JASMIN, G. & CHA, J. W. (1969) Renal adenomas induced in rats by dimethylnitrosamine. An electron microscopic study. *Arch. Pathol.*, **87**: 267–278.

JENISSEN, H., HOSHINO, J., & KRÖGER, H. (1971) [The influence of carcinogenic nitrosamines on the induction of serine dehydratase in the rat liver.] *Z. Krebsforsch.*, **75**: 246 252 (in German).

JOHNSON, J. H. (1966) Internal can corrosion due to high nitrate content of canned vegetables. *Proc. Fla. State Hort. Sci.*, **79**: 239 242.

JUSKIEWICZ, T. & KOWALSKI, B. (1974) *Passage of nitrosamines from rumen into milk in goats.* IARC Sci. Publ. No. 9, pp. 173 176.

KAMM, L., MCKEOWN, G. G., & SMITH, D. M. (1965) New colorimetric method for the determination of the nitrate and nitrite content of baby foods. *J. Assoc. Off. Agric. Chem.*, **48** (5): 892 897.

KAMM, J. J., DASHMAN, T., CONNEY, A. H., & BURNS, J. J. (1973) Protective effect of ascorbic acid on hepatoxicity caused by sodium nitrite and aminopyrin. *Proc. Natl Acad. Sci.*, **70**: 747 749.

KAPELLER-ADLER, R. & KRAEL, J. (1930a) [Research on nitrogen distribution in the muscle of different animal species I.] *Biochem. Z.*, **221**: 437–460 (in German).

KAPELLER-ADLER, R. & KRAEL, J. (1930b) [Research on nitrogen distribution in the muscle of different animal species II.] *Biochem. Z.*, **224**: 364–377 (in German).

KAUDEWITZ, F. (1959) Production of bacterial mutants with nitrous acid. *Nature (Lond.)*, **183**: 1829–1830.

KAWABATA, T. (1974) *Addendum, N-nitroso compounds: Recent studies in Japan.* IARC Sci. Publ. No. 9, pp. 154–158.

KEATING, J. P., LELL, M. E., STRAUSS, A. W., ZARKOWSKY, H., & SMITH, G. E. (1973) Infantile methemoglobinemia caused by carrot juice. *N. Eng. J. Med.*, **288**: 824–826.

KEOHANE, K. W. & METCALF, W. K. (1960) An investigation of the differing sensitivity of juvenile and adult erythrocytes to methemoglobinization. *Phys. med. Biol.*, **5**: 27–35.

94

KILGORE, L., STASCH, A. R. & BARRENTINE, B. F. (1963) Nitrate content of beets, collards, turnip greens. *J. Am. Diet. Assoc.*, **43**: 39–42.

KING, E. J. (1959) *Qualitative analysis and electrolytic solutions.* New York, Harcourt, Brace & Co., pp. 519.

KIRKLAND, D. J. & PICK, C. R. (1973) The histological appearance of tumours derived from rat embryo cells transformed *in vitro* spontaneously and after treatment with nitrosomethylurea. *Br. J. Cancer*, **28**: 440–452.

KLUBES, P. & JONDORF, W. R. (1971) Dimethyl nitrosamine formation from sodium nitrite and dimethylamine by bacterial flora of rat intestine. *Res. Commun. chem. Pathol. Pharmacol.*, **2** (1): 24–34.

KNELSON, J. H. & LEE, R. E. (1977) Oxides of nitrogen in the atmosphere: Origin, fate and public health implications. *Ambio*, **6** (2–3): 126–130.

KNOTEK, Z. & SCHMIDT, P. (1964) Pathogenesis, incidence and possibilities of preventing alimentary nitrate methemoglobinemia in infants. *Pediatrics*, **34**: 78–83.

KOCIBA, R. J. & SLEIGHT, S. D. (1970) Nitrite toxicosis in the ascorbic acid-deficient guinea pig. *Toxicol. appl. Pharmacol.* **16**: 424–429.

KOHL, D. H., SHEARER, G. B., & COMMONER, B. (1971) Fertilizer nitrogen: Contribution to nitrate in surface water in a corn belt watershed. *Science*, **174**: 1331–1334.

KOPPANG, N. (1964) An outbreak of toxic liver injury in ruminants. *Nord. vet. Med.*, **16**: 305–322.

KORDAČ, V., SCHÖN, E., & BRAUN, A. (1969) Hepatocancerogenesis in mice with simultaneous administration of diethylnitrosamine and the virus Motol. *Neoplasma*, **16**: 485–490.

KOYAMA, T., HANDA, J., HANDA, H., & MATSUMOTO, S. (1970) Methylnitrosourea-induced malformations of brain in SD-JCL rat. *Arch. Neurol.*, **22**: 342–347.

KRAVITZ, H., ELEGANT, L. D., KAISER, E., & KAGAN, B. M. (1956) Methemoglobin values in premature and mature infants and children. *A.M.A.J. Dis. Child.*, **91**: 1–5.

KRÖLLER, E. (1967) [Investigations of the evidence of nitrosamines in tobacco smoke and food.] *Dtsch. Lebensm. Rundsch.*, **63** (10): 303–305 (in German).

KRÜGER, F. W. (1972) [On the biochemistry of carcinogenic N-nitroso compounds.] *Ärztliche Forsch.*, **26** (6): 197–202 (in German).

KRÜGER, F. W. (1973) Metabolism of nitrosamines *in vivo*. II. On the methylation of nucleic acids by aliphatic di-n-alkylnitrosamines *in vivo*, caused by beta-oxidation: The increased formation of 7-methyl-guanine after application of beta-hydroxypropyl-nitrosamine compared to that after application of di-N-propyl-nitrosamine. *Z. Krebsforsch.*, **79**: 90–97.

KÜBLER, W. (1958) [The importance of the nitrate content of vegetables in child nutrition.] *Z. Kinderheilk.*, **81**: 405–416 (in German).

KÜBLER, W. (1965) [Methaemoglobinaemia in infants after feeding with spinach.] *Dtsch. med. Wschr.*, **90**: 1881–1882 (in German).

KUNZ, W., SCHAUDE, G., & THOMAS, C. (1969) [The effect of phenobarbital and halogenated hydrocarbons on nitrosamine carcinogenesis.] *Z. Krebsforsch.*, **72**: 291–304 (in German).

KÜNZER, W. & SCHNEIDER, D. (1953) [The activity of the reducing enzyme system in erythrocytes of young infants.] *Acta haemat.*, **9**: 346 (in German).

KUWAHARA, A., OTSUKA, H., & NAGMATSU, A. (1972) Induction of hemangiomatous lesions with dimethylnitrosamine. Influence of route of administration and strain of mice. *Gann*, **63**: 499–502.

LACASSAGNE, A., BUU-HOI, N., GIAO, N. B., & FERRANDO, R. (1968) Retarding effect of resirpine on the cancerization of rat liver induced by diethylnitrosamine. *Bull. Cancer*, **55** (1): 87–89.

LEE, D. H. K. (1970a) Nitrates, nitrites and methemoglobinemia. *Environ. Res.*, **3**: 484–511.

LEE, G. F. (1970b) *Eutrophication.* Occasional Paper No. 2, University of Wisconsin, Water Resources Center, Madison, pp. 12–13.

LEE, K. Y. & LIJINSKY, W. (1966) Alkylation of rat liver RNA by cyclic N-nitrosamines in vivo. J. Natl Cancer Inst., 37 (A): 401–407.

LEHMAN, A. J. (1958) Quarterly report to the editor on topics of current interest. Nitrates and nitrites in meat products. Assoc. Food Drug Off. US Q. Bull., 22: 136–138.

LEMBKE, A. & MOEBUS, O. (1970) Is there a connection between the occurrence of nitrite-producing microorganisms in cheese and the formation of nitrosamine? Proceedings of the 18th International Dairy Congress A.2.3, p. 139.

LIJINSKY, W. (1974) Reaction of drugs with nitrous acid as a source of carcinogenic nitrosamines. Cancer Res., 34: 255 258.

LIJINSKY, W. & EPSTEIN, S. S. (1970) Nitrosamines as environmental carcinogens. Nature (Lond.), 225: 21–23.

LIJINSKY, W. & GREENBLATT, M. (1972) Carcinogen dimethylnitrosamine produced in vivo from nitrite and aminopyrine. Nature (New Biology), 236: 177–178.

LIJINSKY, W. & SINGER, G. (1974) Formation of nitrosamines from tertiary amines and nitrous acid. IARC Sci. Publ. No. 9, pp. 111–114.

LIJINSKY, W., TOMATIS, L., & WENGEN, C. E. M. (1969) Lung tumors in rats treated with N-nitrosoheptamethyleneimine and N-nitrosooctamethyleneimine. Proc. Soc. Exp. Biol. Med., 130: 945 949.

LIJINSKY, W., FERRERO, A., MONTESANO, R., & WENYON, C. E. M. (1970) Tumorigenecity of cyclic nitrosamines in Syrian golden hamsters. Z. Krebsforsch., 74: 185 189.

LIJINSKY, W., CONRAD, E., & VAN DE BOGART, R. (1972a) Carcinogenic nitrosamines formed by drug/nitrite interactions. Nature (Lond.), 239: 165–167.

LIJINSKY, W., CONRAD, E., & VAN DE BOGART, R. (1972b) Formation of carcinogenic nitrosamines by interaction of drugs with nitrite. IARC Sci. Publ. No. 3, pp. 130–133.

LIJINSKY, W., KEEFER, L., CONRAD, E., & VAN DE BOGART, R. (1972c) Nitrosation of tertiary amines and some biologic implications. J. Natl Cancer Inst., 49 (5): 1239–1249.

LIJINSKY, W., GREENBLATT, M., & KOMMINENI, C. (1973a) Brief communication: Feeding studies of nitrilotriacetic acid and derivatives in rats. J. Natl Cancer Inst., 50: 1061–1063.

LIJINSKY, W., KEEFER, L., LOO, J., & ROSS, A. E. (1973b) Studies of alkylation of nucleic acids in rats by cyclic nitrosamines. Cancer Res., 33: 1634–1641.

LIJINSKY, W., TAYLOR, H. W., SNYDER, C., & NETTESHEIM, P. (1973c) Malignant tumors of liver and lung in rats fed aminopyrine or heptamethyleneimine together with nitrite. Nature (Lond.), 244: 176 178.

LOVELESS, A. & HAMPTON, C. (1969) Inactivation and mutation of coliphage T2 by N-methyl- and N-ethyl-N-nitrosourea. Mutat. Res., 7: 1–12.

LUHRS, C. E. (1973) Nitrogenous materials in the environment. Draft report. US Environmental Protection Agency, Hazardous Materials Advisory Committee.

MAEKAWA, H. & ODASHIMA, S. (1975) Induction of tumours of the nervous system in the HCI/N rat with N-buyl-1-nitrosourea administered transplacentally, neonatally or via maternal milk. Gann, 66: 175–183.

MAGEE, P. N. (1956) Toxic liver injury. The metabolism of dimethylnitrosamine. Biochem. J., 64: 676 682.

MAGEE, P. N. (1972) Possibilities of hazard from nitrosamines in industry. Ann. occup. Hyg., 15: 19–22.

MAGEE, P. N. & BARNES, J. M. (1962) Induction of kidney tumours in the rat with dimethyl (N-nitrosodimethylamine) nitrosamine. J. Pathol. Bacteriol., 84: 19–31.

MAGEE, P. N. & BARNES, J. M. (1967) Carcinogenic nitroso compounds. Adv. Cancer Res., 10: 163–246.

MAGEE, P. N., MONTESANO, R., & PREUSSMANN, R. (1976) N-nitroso compounds and related carcinogens. In: Searle, E. ed., Chemical carcinogens. ACS monograph No. 173, pp 491–625.

MAKIURA, S., KAMMAMOTO, Y., SUGIHARA, S., HIRAO, K., HIASA, Y., ARAI, M., & ITO, N. (1973) Effect of 1-naphthyl isothiocyanate and 3-methylcholanthrene on hepato-carcinogenesis in rats treated with diethylnitrosamine. Gann, 64: 101–104.

96

MALINS, D. C., ROUBAL, W. T., & ROBISCH, P. A. (1970) The possible nitrosation of amines in smoked chub. *J. agric. Food. Chem.*, **18** (4): 740 741.

MALLING, H. V. (1966) Mutagenicity of two potent carcinogens dimethylnitrosamine and diethylnitrosamine in *Neurospora crassa*. *Mutat. Res.*, **3**: 537–540.

MANNING, P. B., COULTRER, S. T., & JENNESS, R. (1968) Determination of nitrate and nitrite in milk and dry milk products. *J. Dairy Sci.*, **51**: (11): 1725–1730.

MARCULESCU, V. (1917) Determination of nitrates in water. *Stud. Prot. Epurarea Apelor.*, **15**: 127–161

MARRIOTT, W. M., HARTMANN, A. F., & SENN, M. J. E. (1933) Observations on the nature and treatment of diarrhea and the associated systemic disturbances. *J. Pediatr.*, **3**: 181–191.

MCLEAN, A. E. M. & MAGEE, P. N. (1970) Increased renal carcinogenesis by dimethylnitrosamine in protein deficient rats. *Br. J. exp. Pathol.*, **51**: 587 590.

MCLEAN, A. E. & VERSCHUUREN, H. G. (1969) Effects of diet and microsomal enzyme induction on the toxicity of dimethylnitrosamine. *Br. J. exp. Pathol.*, **50**: 22–25.

METCALF, W. K. (1961) The sensitivity of haemoglobin to oxidation in various conditions. *Nature (Lond.)*, **190**: 543–544.

MIRNA, A. (1974) Determination of free and bound nitrite. In: *Proceedings of the International Symposium on Nitrite in Meat Products, Ziest, 10–14 September 1973*, Wageningen, Centre for Agricultural Publishing and Documentation, pp. 21–28.

MIRVISH, S. S. (1970) Kinetics of dimethylamine nitrosation in relation to nitrosamine carcinogenesis. *J. Natl Cancer Inst.*, **44** (3): 633–639.

MIRVISH, S. S. (1971) Kinetics of nitrosamide formation from alkylureas, *N*-alkylurethanes and alkylguanidines: possible implications for the etiology of human gastric cancer. *J. Natl Cancer Inst.*, **46**: 1183–1193.

MIRVISH, S. S. (1975) Formation of *N*-nitroso compounds: chemistry, kinetics, and *in vivo* occurrence. *Toxicol. appl. Pharmacol.*, **31**: 325–351.

MIRVISH, S. S. (in press) *N*-nitroso compounds, nitrite and nitrate: possible implications for the causation of human cancer. *J. Water Pollut.*

MIRVISH, S., WALLCAVE, L., EAGEN, M., & SHUBIK, P. (1972) Ascorbate-nitrite reaction: possible means of blocking the formation of carcinogenic *N*-nitroso compounds. *Science*, **177**: 65–68.

MIRVISH, S. S., SAMS, J., FAN, T. Y., & TANNENBAUM, S. R. (1973a) Kinetics of nitrosation of the amino acids proline hydroxyproline and sarcosine. *J. Natl Cancer Inst.*, **51**: 1833 1840.

MIRVISH, S. S., CARDESA, A., WALLCAVE, L., & SHUBIK, P. (1973b) Effect on sodium ascorabte on lung adenoma induction by amines plus nitrite. *Proc. Am. Assoc. Cancer Res.*, **14**: 102.

MIRVISH, S. S., ARNOLD, S., CONRAD, E., GHADIRIAN, P., KOMMINENI, V. R. C., & SAMS, J. (1974) *The formation of N-nitroso compounds, preparation of heptafluorobutyryl derivatives of ureas, and the fate of nitrite in the rat stomach.* IARC Sci. Publ. No. 9, pp. 67–70.

MIRVISH, S. S., CARDESA, A., WALLCAVE, L., & SHUBIK, P. (1975) Induction of mouse lung adenomas by amines or ureas plus nitrite and by *N*-nitroso compounds: effects of ascorbates, gallic acid, thyocyanate, and caffeine. *J. Natl Cancer Inst.*, **55**: 633–636.

MIRVISH, S. S., ISSENBERG, P., & SORNSON, H. C. (1976) Air-water and ether-water distribution of *N*-nitroso compounds: implications for laboratory safety, analytical methodology, and carcinogenicity for the rat esophagus, nose, and liver. *J. Natl Cancer Inst.*, **56** (6): 1125–1129.

MÖHLER, K. & MAYRHOFER, O. L. (1968) [Evidence and determination of nitrosamines in food.] *Z. Lebensm. Unter-Forsch.*, **135** (6): 313–318 (in German).

MÖHLER, K. & MAYRHOFER, O. L. (1969) [Effect of various factors on the formation of nitrosamines in meat products.] *15th European Meeting of the Meat Research Workers, Helsinki, 17–24 August 1969*, pp. 302–304 (in German).

MOHR, U. & HILFRICH, J. (1972) Brief communication: Effect of a single dose of *N*-diethylnitrosamine on the rat kidney. *J. Natl Cancer Inst.*, **49**: 1729–1731.

97

MONTESANO, R. (1970) Systemic carcinogens (N-nitroso compounds) and synergistic or additive effects of respiratory carcinogenesis. *Tumori*, **56:** 335 344.

MONTESANO, R. & BARTCH, H. (1976) Mutagenic and carcinogenic N-nitroso compounds: possible environmental hazards. *Mutat. Res.*, **32:** 179–228.

MONTESANO, R. & SAFFIOTTI, U. (1968) Carcinogenic response of the respiratory tract of Syrian golden hamsters to different doses of diethylnitrosamine. *Cancer Res.*, **28:** 2197–2210.

MOTYLEV, V. D. (1969) [The question of age-related methaemoglobinaemia caused by nitrates and nitrites and its prophylaxis.] Author's summary of a thesis for the degree of Candidate of Medical Sciences, Leningrad, (in Russian).

MURTHY, Y. K. S., THIEMANN, J. E., CORONELLI, C. I., & SENSI, P. (1966) Alanosine, a new antiviral and antitumour agent isolated from a streptomyces. *Nature (Lond.)*, **211:** 1198 1199.

MYSLIWY, T. S., WICK, E. L., ARCHER, M. C., SHANK, R. C., & NEWBERNE, P. M. (1974) Formation of N-nitrosopyrrolidine in a dog's stomach. *Br. J. Cancer*, **30:** 279–283.

NAGATA, Y. & MIRNA, A. (1974) [Influence exerted by processing upon the formation of nitrosamines in meat products.] *Fleischwirtschaft.*, 1781–1788 (in German).

NAPALKOV, N. P. (1971) [Experimentation on transplacental blastomogenesis as a tool for study of etiopathogenesis of tumours in children.] *Vop. Onkol.*, **17** (8): 3 15 (in Russian).

NASHED, N. & JUBBUR, G. (1966) A genetic and functional characterization of adenine mutants induced in yeast by 1-nitroso-imidazolidone-2 and nitrous acid. *Z. Vererbungsl.*, **98:** 106–110.

NEURATH, H. (1972) *Nitrosamine formation from precursors in tobacco smoke*. IARC Sci. Publ. No. 3, pp. 134–136.

NEURATH, G. & SCHREIBER, O. (1974) *Investigations on amines in the human environment*. IARC Sci. Publ. No. 9, pp. 211 214.

NEURATH, G., PIRMANN, B., & WICHERNOTT, (1964) [On the question of nitroso compounds in tobacco smoke.] *Beitr. Tabakforsch.*, **2** (7): 311–319 (in German).

NEWBERNE, P. M. & SHANK. R. C. (1973) Induction of liver and lung tumors in rats by the simultaneous administration of sodium nitrite and morpholine. *Food Cosmet. Toxicol.*, **11:** 819–825.

NEWELL, J. E. & SISKEN, H. R. (1972) Determination of nitrosodimethylamine in the low parts per billion. *J. agric. Food Chem.*, **20** (3): 711–713.

NIEHS (1970) *Nitrates, nitrites and methemoglobinemia*. North Carolina, National Institute of Environmental Health Sciences, pp. 43 (Rep. No. 2).

NOROHNA, R. F. & GOODALL, C. M. (1972) Nasal tumors in starved rats injected once with dimethylnitrosamine. *N.Z. med. J.*, **75:** 374 375.

NYE, J. C. (1973) *Nitrogenous compounds in the environment*. Draft Report, Washington, DC, US Environmental Protection Agency, Hazardous Materials Advisory Committee. pp. 95–109.

O'CONNOR, P. J., CAPPS, M. J., & CRAIG, A. W. (1973) Comparative studies of the hepatocarcinogen N,N-dimethylnitrosamine *in vivo*: reaction sites in rat liver DNA and the significance of their relative stabilities. *Br. J. Cancer*, **27:** 153 166.

ODASHIMA, S. (1969) Experimental carcinoma of the glandular stomach in rats. 1. Effect of 7, 12-dimethylbenz[a]anthracene or 4-nitroquinolene 1-oxide placed on glandular stomach combined with oral administration of N,N^1-(2,7-fluorenylene) bisacetamide or N-nitrosodiethylamine. *Gann*, **60:** 211–222.

OEHME, F. W. (1975) Vetinary toxicology. In: Caserett, L. J. & Doulle, J., ed. *Toxicology, the basic science of poisons*. New York, Macmillan Publishing Co., Inc., pp. 701–727.

OKADA, M. & SUZUKI, E. (1972) Metabolism of butyl (4-hydroxybutyl) nitrosamine in rats. *Gann*, **63** (3): 391–392.

OKADA, M., SUZUKI, E., ANJYO, T., & MOCHIZUKI, M. (1975) Mutagenicity of γ-acetoxydialkylnitrosamines: model compounds for an ultimate carcinogen. *Gann*, **66** (4): 457–458.

OKAJIMA, E., HIRAMATSU, T., MOTOMIYA, Y., IRWA, K., IJUIN, M., & ITO, N. (1971) Effect

of DL-tryptophan on tumorigenesis in the urinary bladder and liver of rats treated with *N*-nitrosodibutylamine. *Gann*, **62**: 163–169.

OLSON, O. E., NELSON, D. L., & EMERICK, R. J. (1963) Effect of nitrate and some of its reduction products on carotine stability. *J. agric. Food Chem*, **11**: 140–143.

ORGERON, J. D., MARTIN, J. D., CARAWAY, C. T., MARTINE, R. M., & HAUSER, G. H. (1957) Methemoglobinemia from eating meat with high nitrite content. *Public Health Rep.*, **72** (3): 189–193.

OSSKE, G., WARZOK, R., & SCHNEIDER, J. (1972) [Transplacental tumour induction by endogenous *N*-ethyl-*N*-nitrosourea in the rat.] *J. Arch. Geschwulstforsch.*, **40**: 244–247 (in German).

OWENS, M. (1970) Nutrient balances in rivers. *Proc. Soc. Water Treat. Exam.*, **19**: 239–247.

PANALAKS, T., IYENGAR, J. R., & SEN, N. P. (1972) Nitrate, nitrite and dimethylnitrosamine in cured meat products. *J. Assoc. Off. Anal. Chem.*, **56** (3): 621–625.

PANALAKS, T., IYENGAR, J. R., DONALDSON, B. A., MILES, W. F., & SEN, N. P. (1974) Further survey of cured meat products for volatile *N*-nitrosamines. *J. Assoc. Off. Anal. Chem.*, **57** (4): 806–812.

PASTERNAK, L. (1962) [Mutagenic effects of dimethylnitrosamine with *Drosophila melanogaster*.] *Naturwiss.*, **16**: 381 (in German).

PASTERNAK, L. (1964) Investigation into the mutagenic effect of several nitrosamine and nitrosamide compounds. *Arzneimittel-Forsch.*, **14**: 802–804.

PENSABENE, J. W., FIDDLER, W., GATES, R. A., FAGAN, J. C., & WASSERMAN, A. E. (1974) Effect of frying and other cooking conditions on nitrosopyrrolidine formation in bacon. *J. Food Sci.*, **39**: 314–136.

PERRY, T. L., SHAW, K. N. F., WALKER, D., & REDLICH, D. (1962) Urinary excretion of amines in normal children. *Pediatrics*, **30**: 576–584.

PETUKHOV, N. I., RYVKIN, A. I., GAINULLEN, G. G., & LANDYSHEVA, V. I. (1972) [Methaemoglobinaemia in children and adults caused by nitrates in water] *Gig. i Sanit.*, **3**: 14–17 (in Russian).

PETUKHOV, N. I. & IVANOV, A. V. (1970) Investigation of certain psychophysiological reactions in children suffering from methemoglobinemia due to nitrates in water. *Gig. i Sanit.*, **35** (1): 26–28.

PHILLIPS, J. C., LAKE, B. G., HEADING, C. E., GANGOLLI, S. D., & LLOYD, A. G. (1975a) Studies on the metabolism of dimethylnitrosamine in the rat. I. Effect of dose, route of administration and sex. *Food Cosmet. Toxicol.*, **13**: 203–209.

PHILLIPS, J. C., HEADING, C. E., LAKE, B. G., GANGOLLI, S. D., & LLOYD, A. G. (1975b) Studies on the metabolism of dimethylnitrosamine in the rat. II. The effects of phenobarbitone and 20-methylcholanthrene on the *in vitro* and *in vivo* metabolism and acute toxicity of dimethylnitrosamine in young and mature rats. *Food Cosmet. Toxicol.*, **13**: 611–617.

PHILLIPS, W. E. J. (1966) Effect of dietary nitrite on the liver storage of vitamin A in the rat. *Can. J. Biochem.*, **44** (1): 1–7.

PHILLIPS, W. E. J. (1968a) Changes in the nitrate and nitrite contents of fresh and processed spinach during storage. *J. agric. Food Chem.*, **16** (1): 88–91.

PHILLIPS, W. E. J. (1968b) Nitrate content of foods — public health implications. *Can. Inst. Food Technol. J.*, **1** (3): 98–103.

PHILLIPS, W. E. J. (1969) Lack of nitrite accumulation in partially consumed jars of baby food. *Can. Inst. Food Technol. J.*, **2** (4): 160–161.

PHILLIPS, W. E. J. (1971) Naturally occurring nitrate and nitrite in foods in relation to infant methemoglobinemia. *Food Cosmet. Toxicol.*, **9**: 219–228.

PIELSTICKER, K. (1967) Absence of malformations following diethylnitrosamine in the golden hamster. *Naturwiss.*, **54**: 340–341.

PISCIOTTA, A. V., EBBE, S. N., & HINZ, J. E. (1959) Clinical and laboratory features of two variants of methemoglobin M disease. *J. Lab. clin. Med.*, **54**: 73–87.

PITTS, J. N. Jr. & LLOYD, A. C. (1973) Discharges into the atmosphere. In: *Nitrogenous compounds in the environment.* Washington, US Environmental Protection Agency, pp. 43–65 (Rep No. EPA-SAB-73-001).

PLATT, D. & HERING, F. J. (1973) The effect of Calciparin on diethylnitrosamine-induced liver tumors in the rat. *Arzneimittelforsch.*, **23** (7): 956–961.

POUND, A. W. LAWSON, T. A., & HORN. L. (1973) Increased carcinogenic action of dimethylnitrosamine after prior administration of carbon tetrachloride. *Br. J. Cancer,* **27:** 451–459.

PREDA, N., POPA, L., GALEA, V., & SIMU, G. (1976) *N*-nitroso compound formation by chlordiazepoxide and nitrite interaction *in vitro* and *in vivo*: protective action of ascorbic acid. IARC Sci. Publ. No. 14, pp. 301–304.

PREUSSMAN, R. (1975) [Carcinogenic chemicals in the human environment.] *Handbuch der Allg. Pathol.,* VI/6/11. 448–482 (in German).

PURCHASE, I. F. H., TUSTIN, R. C., & van RENSBERG. S. J. (1975) Biological testing of food grown in the Transkei. *Food Cosmet. Toxicol.,* **13:** 639–647.

QUISPEL, A., ed. (1974) – *The biology of nitrogen fixation.* Amsterdam, North Holland Publishing Company.

RABES, H., HARTENSTEIN, R., & SCHOLZE, P. (1970) Specific stages of cellular response to homeostatic control during diethylnitrosamine–induced liver carcinogenesis. *Experientia (Basel),* **26:** 1356–1359.

RABES, H., HARTENSTEIN, R., & GMINDER, J. (1971) Kidney neoplasms induced by diethylnitrosamine in partially hepatectomized rats. *Naturwiss.,* **58:** 102–103.

RAMMELL, C. G. & JOERIN, M. N. (1972) Determination of nitrite in cheese and other dairy products. *J. Dairy Res.,* **39:** 89–94.

REDDY, B. S. & THOMAS, J. W. (1962) Interrelationship between thyroid status and nitrate on carotine conversion. *J. anim. Sci.,* **21:** 1010.

REEVES, M. J., BIRTLES, A. B., COURCHE, R. C., & ALDRICK, R. J. (1974) *Groundwater resources of the Vale of York.* London, Water Resources Board Report, pp. 90.

RHOADES, J. W. & JOHNSON. D. E. (1970) Gas-chromatography and selective detection of *N*-nitrosamines. *J. Chromatogr. Sci.,* **8:** 616–618.

RICHARDSON, W. D. (1907) Nitrates in vegetable foods in cured meats and elsewhere. *J. Am. Chem. Soc.,* **29:** 1757–1767.

RIDDER, W. E. & OEHME, F. W. (1974) Nitrates as an environmental, animal, and human hazard. *Clin. Toxicol.,* 7 (2): 145–159.

RIEDEL, B. & PIPER, R. (1973) [Early changes of the rat bladder mucosa after experimental cancerogenesis with *N*-butyl-*N*-butanol (4)-nitrosamine.] (author's transl.). *Urol. Int.,* **28:** 322–327.

ROBERTS, J. D. & CASERIO, M. C. (1964) *Basic Principles of Organic Chemistry,* New York, Benjamin, pp. 656–666.

ROBERTS, W. K. & SELL, J. L. (1963) Vitamin A destruction by nitrite *in vitro* and *in vivo. J. anim. Sci.,* **22:** 1081–1085.

ROBERTSON, H. E. & DRAYCOTT, M. E. (1948) *Nitrate poisoning of infants by contaminated drinking water.* Abstracts of papers presented at the fifteenth Annual Christmas Meeting of the Laboratory Section, Canadian Public Health Association, London, Ontario, 13–14 December, p. 30.

ROBERTSON, H. E. & RIDDELL, W. A. (1949) Cyanosis of infants produced by high nitrate concentration in rural waters of Saskatchewan. *Can. J. Public Health,* **40:** 72–77.

ROBINSON, E. & ROBBINS, R. C. (1972) Emissions, concentrations and fate of gaseous atmospheric pollutants. In: Strauss, W., ed. *Air pollution control Part II,* New York, Wiley-Interscience, pp. 1–93.

ROLLER, P. P. & KEEFER, L. K. (1974) *Catalysis of nitrosation reactions by electrophilic species.* IARC Sci. Publ. No. 9, pp. 86–89.

ROSS, J. D. (1963) Deficient activity of DPNH-dependent methemoglobin diaphorase in cord blood erythrocytes. *Blood,* **21** (1): 51–62.

ROSS, J. D. & DESFORGES, J. F. (1959) Reduction of methemoglobin by erythrocytes from cord blood. *Pediatrics,* **23:** 718–726.

SAKSHAUG, J., SÖGNEN, E., HANSEN, M. A., & KOPPANG, N. (1965) Dimethylnitrosamine;

its hepatotoxic effect in sheep and its occurrence in toxic batches of herring meal. *Nature (Lond.)*, **206**: 1261–1262.

SANDER, J. (1971a) [Investigations on the formation of carcinogenic nitroso compounds in the stomach of experimental animals and its significance for man. I] *Arzneimittel-Forsch.*, **21**: 1572–1580 (in German).

SANDER, J. (1971b) [Investigations on the formation of carcinogenic nitroso compounds in the stomach of experimental animals and its significance for man.] *Arzneimittel-Forsch.*, **21**: 1703–1707 (in German).

SANDER, J. (1971c) [Further experiments on tumour induction by oral administration of small doses of *N*-methylbenzylamine and sodium nitrite.] *Z. Krebsforsch.*, **76**: 93–96 (in German).

SANDER, J. & BÜRKLE, G. (1969) [Induction of malignant tumors in rats by simultaneous feeding of nitrite and secondary amines.] *Z. Krebsforsch.*, **73**: 54–66 (in German).

SANDER, J. & SEIF, F. (1969) [Bacterial reduction of nitrate in the stomach of man as a cause of nitrosamine formation.] *Arzneimittel-Forsch.*, **19**: 1091–1093 (in German).

SANDER, J. & SCHWEINSBERG, F. (1972) In vivo *and* in vitro *experiments on the formation of* N-*nitroso compounds from amines or amides and nitrate or nitrite.* IARC Sci. Publ. No.3, pp. 97–103.

SANDER, J. & SCHWEINSBERG, F. (1973) [Tumor induction in mice with methylbenzylnitrosamine in low doses.] *Z. Krebsforsch.*, **79**: 157–161 (in German).

SANDER, J., SCHWEINSBERG, F., & MENZ, H. P. (1968) [Investigations on the formation of carcinogenic nitrosamine in the stomach.] *Hoppe-Seyler's Z. physiol. Chem.*, **349**: 1691–1697 (in German).

SANDER, J., BÜRKLE, G., FLOHE, L., & AEIKENS, B. (1971) [*In vitro* investigations on the possibility of the formation of carcinogenic nitrosamide in the stomach.] *Arzneim-Forsch.*, (Drug Res.), **21** (3): 411–414 (in German).

SANDER, J., LABAR, J., LADENSTEIN, M., & SCHWEINSBERG, F. (1974a) *Quantitative measurement of* in vivo *nitrosamine formation.* IARC Sci. Publ. No. 9, pp. 123–131.

SANDER, J., LABAR, J., LADENSTEIN, M., & SCHWEINSBERG, F. (1974b) *Experiments on the degradation of nitrosamines in plants.* IARC Sci. Publ. No. 9, pp. 205–210.

SATTELMACHER, P. G. (1962) [Methemoglobinemia from nitrates in drinking water.] *Schriftenr. Ver. Wasser Boden. Lufthyg.*, No. 21 (in German).

SAWYER, C. N. (1947) Fertilization of lakes by agricultural and urban drainage. *J. New England Water Works Assoc.*, 109–127.

SCANLAN, R. A. (1975) Nitrosamines in food. *CRC Critical Reviews in Food Technology*, April 1975, pp. 357–402.

SCHERER, E., HOFFMAN, M., EMMELOT, P., & FRIEDRICH-FRESKA, M. (1972) Quantitative study on foci of altered liver cells induced in the rat by a single dose of diethylnitrosamine and partial hepatectomy. *J. Natl Cancer Inst.*, **49**: 93–106.

SCHEUNIG, G. & ZIEBARTH, D. (1976) *Formation of nitrosamines by interaction of some drugs with nitrite in human gastric juice.* IARC Sci. Publ. No. 14, pp. 269–277.

SCHMÄHL, D. (1963) [The origin, growth and chemotherapy of malignant tumours.] *Arzneimittel-Forsch.*, **13** (Supplement): 612 (in German).

SCHMÄHL, D. (1970) [Experimental studies on syncarcinogenesis VI: The addition of minimal doses of four different hepatotropic carcinogens in liver cancer induction in the rat.] *Z. Krebsforsch.*, **74**: 457–466 (in German).

SCHMÄHL, D. & STACKELBERG, S. VON (1968) [The influence of lactoflavin, nicotinamide or dipyridamole on the carcinogenic activity of diethylnitrosamine in rats.] *Arzneimittel-Forsch.*, **18**: 318–320 (in German).

SCHMÄHL, D. & THOMAS, C. (1962) [Acute nitrosomethylurethane poisoning in rats following oral application.] *Arzneimittel-Forsch.*, **12**: 585–587 (in German).

SCHMÄHL, D., THOMAS, C., & KÖNIG, K. (1963) [Studies of syncarcinogenesis: I. Experiments on the induction of cancer in rats by the simultaneous administration of diethylnitrosamine and 4-dimethylamino-azobenzene.] *Z. Krebsforsch.*, **65**: 351–377 (in German).

SCHMÄHL, D., THOMAS, C., SATTLER, W., & SCHIELD, G. F. (1965) [Experimental studies of

syncarcinogenesis: III. Attempts to induce cancer in rats by administering diethyl-nitrosamine and CCl_4 (or ethyl alcohol) simultaneously. In addition, an experimental contribution regarding "alcoholic cirrhosis".] *Z. Krebsforsch.*, **66**: 526-532 (in German).

SCHMÄHL, D., STUTZ, E., & THOMAS, C. (1966) [Experimental studies on syncarcinogenesis. V. Experiments on cancer generation in rats with the simultaneous application of X-ray radiation and diethylnitrosamine or 4-dimethyl-aminobiphenyl.] *Z. Krebsforsch.*, **68**: 68-72 (in German).

SCHMÄHL, D., WAGNER, R., & SCHERF, H. R. (1971) [Influence of immunosuppressive drugs on cancer formation in rat liver by diethylnitrosamine and on the induction of fibrosarcoma by 3,4-benzopyrene.] *Arzneimittel-Forsch.*, **21** (3): 403-404 (in German).

SCHMIDT, P. & KNOTEK, Z. (1970) *Epidemiological evaluation of nitrates as ground water contaminants in Czechoslovakia* (Paper presented to the Sixth International Water Pollution Research Conference, San Francisco).

SCHMID-RUPPIN, K. H. & PAPADOUPOULU, G. (1972) [Effect of diethylnitrosamine (DENA) and influenza viruses on the induction of lung carcinomas in mice.] *Z. Krebsforsch.*, **77**: 150-154 (in German).

SCHMITZ-MOORMAN, P., GEDIGK, P., & DHARAMADHACH, A. (1972) [Early histological and histochemical changes during experimental production of liver carcinoma by die-thylnitrosamine.] *Z. Krebsforsch.*, **77**: 9-16 (in German).

SCHNEIDER, N. R. & YEARY, R. A. (1973) Measurement of nitrite and nitrate in blood. *Am. J. vet. Res.*, **34** (1): 133-136.

SCHOENTAL, R. (1960) Carcinogenic action of diagomethane and of nitroso-*N*-methylu-rethane. *Nature (Lond.)*, **188**: 420-421.

SCHREIBER, H., NETTESHEIM, P., LIJINSKY, W., RICHTER, C. B., & WALBURG, H. E. (1972) Induction of lung cancer in germfree, specific-pathogen-free and infected rats by *N*-nitrosoheptamethyleneimine. Enhancement by respiratory infection. *J. Natl Cancer Inst.*, **49**: 1107-1114.

SCHUPHAN, W. (1965) [The nitrate content of spinach (*Spinacia oleracea, L*) in relation to methaemoglobinaemia in children.] *Z. Ernähr. Wiss.*, **5** (3-4): 207-209 (in German).

SCHUPHAN, W. (1969) [The formation of nitrate and nitrite in plant metabolism.] *Nutritio Dieta*, **11**: 120-132 (in German).

SCHWEINSBERG, F. & SANDER, J. (1972) [Carcinogenic nitrosamine from simple aliphatic tertiary amines and nitrite.] *Hoppe-Seyler's Z. physiol. Chem.*, **353**: 1671-1676 (in German).

SEBRANEK, J. G., CASSENS, R. G., & HOEKSTRA, W. G. (1974) Fate of added nitrite. In: *Proceedings of the International Symposium on Nitrite in Meat Products, Ziest, 10-14 September, 1973*, Wageningen, Centre for Agricultural Publishing and Documenta-tion, pp. 139-147.

SEHGAL, O. P. & KRAUSE, G. F. (1968) Efficiency of nitrous acid as an inactivating and mutagenic agent of intact tobacco mosaic virus and its isolated nucleic acid. *J. Virol.*, **2** (10): 966-971.

SELENKA, F. (1970) Formation and reduction of nitrite in infant nutrients containing nitrate. *Archiv. Hyg.*, **154** (4): 336-345.

SELL, J. L. & ROBERTS, W. K. (1963) Effects of dietary nitrite on the chick: Growth, liver vitamin A stores and thyroid weight. *J. Nutr.*, **79**: 171-178.

SEN, N. P. (1970) Gas-liquid chromatographic determination of dimethylnitrosamine as dimethylnitramine at picogram levels. *J. Chromatogr.*, **51**: 301-304.

SEN, N. P. (1972) The evidence for the presence of dimethylnitrosamine in meat products. *Food Cosmet. Toxicol.*, **10**: 219-223.

SEN, N. P. (1974) *Nitrosamines in toxic constituents in animal foodstuffs*. New York, Academic Press Inc., pp. 131.

SEN, N. P. & DALPE, C. (1972) A simple thin-layer chromatographic technique for the semi-quantitative determination of volatile nitrosamines in alcoholic beverages. *Analyst*, **97**: 216-220.

SEN, N. P. & DONALDSON, B. (1974) *The effect of ascorbic acid and glutathiane on the*

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formation of nitrosopiperazines from piperazine adipate and nitrite. IARC Sci. Publ. No. 9, pp. 103 106.

SEN, N. P., SMITH, D. C., SCHWINGHAMER, L., & MARLEAU, J. J. (1969a) Diethylnitrosamine and other *N*-nitrosamines in foods. *J. Assoc. Off. Anal. Chem.*, **52** (1): 47–52.

SEN, N. P., SMITH, D. C., & SCHWINGHAMER, L. (1969b) Formation of *N*-nitrosamines from secondary amines and nitrite in human and animal gastric juice. *Food Cosmet. Toxicol.*, 7: 301 -307.

SEN, N. P., SMITH, D. C., SCHWINGHAMER, L., & HOWSAM, B. (1970) Formation of nitrosamines in nitrite-treated fish. *Can. Inst. Food Technol. J.*, 3 (2): 66 -69.

SEN, N. P., SCHWINGHAMER, L. A., DONALDSON, B. A., & MILES, W. F. (1972) *N*-nitrosodimethylamine in fish meal. *J. agric. Food Chem.*, **20** (6): 1280–1281.

SEN, N. P., DONALDSON, B., IYENGAR, J. R., & PANALAKS, T. (1973a) Nitrosopyrrolidine and dimethylnitrosamine in bacon. *Nature (Lond.)*, **241**; 473–474.

SEN, N. P., MILES, W. F., DONALDSON, B., PANALAKS, T., & IYENGAR, J. R. (1973b) Formation of nitrosamines in a meat curing mixture. *Nature (Lond.)*, **245**: 104–105.

SEN, N. P., IYENGAR, J. R., DONALDSON, B. A., & PANALAKS, T. (1974a) Effect of sodium nitrite concentration on the formation of nitrosopyrrolidine and dimethylnitrosamine in fried bacon. *J. agric. Food Chem.*, **22** (3): 540–541.

SEN, N. P., DONALDSON, B., CHARBONNEAU, C., & MILES, W. F. (1974b) Effect of additives on the formation of nitrosamines in meat curing mixtures containing spices and nitrite. *J. agric. Food Chem.*, **22** (6): 1125–1130.

SEN, N. P., IYENGAR, J. R., MILES, W. F., & PANALAKS, T. (1976) *Nitrosamines in cured meat products.* IARC Sci. Publ. No. 14, pp. 333–342.

SERFONTEIN, W. J. & HURTER, P. (1966) Nitrosamines as environmental carcinogens. II. Evidence for the presence of nitrosamines in tobacco smoke condensate. *Cancer Res.*, **26**: 575–579.

DE SERRES, F. J., BROCKMAN, H. E., BARNETT, W. E., & KOLMARK, H. G. (1967) Allelic complementation among nitrous acid-induced *ad-3B* mutants of *Neurospora crassa. Mutat. Res.*, **4**; 415–424.

SHANK, R. C. (1975) Toxicology of *N*-nitroso compounds. *Toxicol. appl. Pharmacol.*, **31**: 361 -368.

SHANK, R. C. & NEWBERNE, P. M. (1972) Nitrite-morpholine induced hepatomas. *Food Cosmet. Toxicol.*, **10**: 887 -888.

SHEARER, L. A., GOLDSMITH, J. R., YOUNG, C., KEARNS, O. A., & TAMPLIN, B. R. (1972) Methaemoglobin levels in infants in an area with high nitrate water supply. *Am. J. Public Health,* **62** (9): 1174 1180.

SCHECHTER, H., GARDNER, N., & SHUVAL, H. I. (1972) A micromethod for the determination of nitrite in blood. *Anal. Chem. Acta.*, **60**: 93 -99.

SHUVAL, H. I. & GRUENER, N. (1972) Epidemiological and toxicological aspects of nitrates and nitrites in the environment. *Am. J. Public Health,* **62**: 1045–1052.

SIBAY, T. M. & HAYES, J. A. (1969) Potential carcinogenic effect of streptozotocin. *Lancet,* II: 912.

SIBERT, D. & EISENBRAND, G. (1974) Induction of mitotic gene conversion in *Saccharomyces cerevisae* by *N*-nitrosated pesticides. *Mutat. Res.*, **22**: 121–126.

SIDDIQI, O. H. (1962) Mutagenic action of nitrous acid on *Asperigillus nidulans. Genet. Res.*, **3** (2): 303–314.

SIMON, C. (1966) Nitrite poisoning from spinach. *Lancet,* I (7442): 872.

SIMON, C., MANZKE, H., KAY, H., & MROWETZ, G. (1964) On the occurrence, pathogenesis, and possibilities for prophylaxis of the methemoglobinemia caused by nitrite. *Z. Kinderheilkunde,* **91**: 124–138.

SIMONEIT, B. R. & BURLINGAME, A. L. (1971) Organic analyses of selected areas of Surveyor III recovered on the Apollo 12 mission. *Nature (Lond.)*, **234**: 210–211.

SINGLEY, T. L. (1962) Secondary methemoglobineamia due to the adulteration of fish with sodium nitrite. *Ann. internal Med.*, **57** (5): 800–803.

SINHA, D. P. & SLEIGHT, S. D. (1971) Pathogenesis of abortion in acute nitrite toxicosis in guinea pigs. *Toxicol. appl. Pharmacol.*, **18** (2): 340–347.

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SINIOS VON, A. & WODSAK, W. (1965) [Spinach toxicity in children.] *Dtsch Med. Wochenschr.*, **90**: 1856 1863 (in German).

SKRIVAN, J. (1971) Methemoglobinemia in pregnancy. *Acta Universitatis Carolinae Medica*, **17**: 123-160.

SLEIGHT, S. D. & ATALLAH, O. A. (1968) Reproduction in the guinea pig as affected by chronic administration of potassium nitrate and potassium nitrite. *Toxicol. appl. Pharmacol.*, **12**: 179-185.

SMITH, C. H. (1972) Polycythemia, methemoglobinemia, sulfhemoglobinemia, and miscellaneous anemias In: *Blood diseases of infancy and childhood*, 3rd ed., Saint Louis, The C. V. Mosby Company, pp. 450 454.

SMITH, G. E. (1964) Nitrate problems in plants and water supplies in Missouri. In: *2nd Annual Symposium on Relation of Geology and Trace Elements to Nutrition.*

SMITH, G. E. (1966) Causes of nitrate accumulation in plants and water supplies. In: *18th Annual Midwest Fertilizer Conference, Chicago, Illinois.*

SMITH, J. E. & BEUTLER, E. (1966) Methemoglobin formation and reduction in man and various animals species. *Am. J. Physiol.*, **210**: 347 350.

SMITH, P. A. S. & LOEPPKY, R. N. (1967) Nitrosative cleavage of tertiary amines. *J. Am. Chem. Soc.*, **89** (5): 1147 1157.

SMITH, R. P. (1969) The significance of methaemoglobinaemia in toxicology. In: Blood, F. R., ed. *Essays in toxicology Volume I*, New York and London, Academic Press, pp. 84 113.

SMITH, R. P. (1975) Toxicology of the formed elements of the blood. In: Casseret, L. J. & Doull, J., ed. *Toxicology the basic science of poisons.* New York, Macmillan Publishing Co., Inc., pp. 225 243.

SPENCER, R. (1967) *A study of factors affecting the quality and shelf life of vacuum packed bacon, and of the behaviour of Wiltshire cooked bacon packed and stored under controlled conditions.* (BFMIRA Res. Rep. No. 136).

SPIEGELHALDER, B., EISENBRAND, G., & PREUSSMAN, R. (1976) The influence of dietary intake of nitrate on the nitrite content in human saliva: a factor of possible relevance for *in vivo* formation of *N*-nitroso compounds. *Food Cosmet. Toxicol.*, **14** (6): 545–548.

STANDFORD, G., ENGLAND, C. B., & TAYLOR, A. W. (1969) *Fertilizer use and Water quality*, US Department of Agriculture, ARS. pp. 41–168.

STEINBERG, R. A. & THOM, C. (1940) Mutations and reversions in reproductivity of Aspergilli with nitrite, colchicine and d-lysine. *Proc. Natl Acad. Sci.*, **26** (6): 363–366.

STENBACK, F. G., FERRERO, A., & SHUBIK, P. (1973) Synergistic effects of diethylnitrosamine and different dusts on respiratory carcinogenesis in hamsters. *Cancer Res.*, **33**: 2209–2214.

STEPHANY, R. W., FREUDENTHAL, J., & SCHULLER, P. L. (1976) *Quantitative and qualitative determination of some volatile nitrosamines in various meat products,* IARC Sci. Publ. No. 14, pp. 343–354.

STEWART, B. W., SWANN, P. F., HOLSMAN, J. W., & MAGEE, P. N. (1974) Cellular injury and carcinogenesis. Evidence for the alkylation of rat liver nucleic acids *in vivo* by *N*-nitrosomorpholine. *Z. Krebsforsch.*, **82** (1): 1 12.

STOEWSAND, G. S. (1973) Nitrite-induced methemoglobinemia in guinea pigs: Influence of diets containing beets with varying amounts of nitrate, and the effect of ascorbic acid, and methionine. *J. Nutr.*, **103** (3): 419 424.

STONE, I. M., LASCHIVER, C., & SALETAN, L. T. (1968) Nitrates in worts, beers and brewing. *Waller Stein Lab. Communications*, **31** (106): 193–201.

STRACK, H. B., FREESE, E. B., & FREESE, E. (1964) Comparison of mutation and inactivation rates induced in bacteriophage and transforming DNA by various mutagens. *Mutat. Res.*, **1**: 10-21.

STRACKS W. & FERON, V. J. (1973) Ultrastructure of pulmonary adenomas induced by intratracheal instillation of diethylnitrosamine in Syrian golden hamsters. *Eur. J. Cancer*, **9**: 359–362.

SVOBODA, D. & HIGGINSON, J. (1968) A comparison of ultrastructural changes in rat liver due to chemical carcinogens. *Cancer Res.*, **28**: 1703-1733.

SWANN, P. F. & KAUFMAN, D. G. (1973) The dose response for the induction of kidney tumors in the rat by a single dose of dimethylnitrosamine. *Br. J. Cancer*, **28**: 83–84.

SWANN, P. F. & MAGEE, P. W. (1971) Nitrosamine-induced carcinogenesis: The alkylation of N-7 of guanine of nucleic acids of the rat by diethylnitrosamine. *N*-ethyl-*N*-nitrosourea and ethylmethanesulphonate. *Biochem. J.*, **125**: 841–847.

SWENBERG, J. A., KOESTNER, A., WESCHLER, W., & DENLINGER, R. H. (1972) Quantitative aspects of transplacental tumor induction with ethylnitrosourea in rats. *Cancer Res.*, **32**: 2656–2660.

SYLVESTER, R. O. (1961) Nutrient content of drainage water forested, urban and agricultural areas. In: *Algae and Metropolitan Wastes. Transactions of the 1960 Seminar*, Robert A. Taft Sanitary Engineering Center, pp. 80–87, (Technical Report W61 3).

TAKAYAMA, S. (1968) The histological and autoradiographical studies of mouse liver during the course of carcinogenesis by dimethylnitrosamine. *Z. Krebsforsch.*, **71**: 246–254.

TAKAYAMA, S. & IMAIZUMI, T. (1969) Sequential effects of chemically different carcinogens, dimethylnitrosamine and 4-dimethylaminoazobenzene, on hepatocarcinogenesis in rats. *Int. J. Cancer*, **4**: 373 383.

TAKAYAMA, S. & MURAMATSU, M. (1969) Incorporation of tritiated dimethylnitrosamine into subcellular fractions of mouse liver after long-term administration of dimethylnitrosamine. *Z. Krebsforsch.*, **73**: 172–179.

TANNENBAUM, S. R., SINSKEY, A. J., WEISMAN, M., & BISHOP, W. (1974) Nitrite in human saliva. Its possible relationship to nitrosamine formation. *J. Natl Cancer Inst.*, **53** (1): 79–84.

TANNENBAUM, S. R., WEISMAN, M., & FELT, D. (1976) The effect of nitrate intake on nitrite formation in human saliva. *Food Cosmet. Toxicol.*, **14** (6): 549 552.

TAYLOR, H. W. & LIJINSKY, W. (1975) Tumour induction in rats by feeding heptamethyleneimine and nitrite in water. *Cancer Res.*, **35**: 812–815.

TELLING G. M. (1972) A gas-liquid chromatographic procedure for the detection of volatile *N*-nitrosamines at the 10 parts per billion level in foodstuffs after conversion to their corresponding nitramines. *J. Chromatogr.*, **73**: 79–87.

TELLING, G. M., BRYCE, T. A., & ALTHORPE, J. (1971) Use of vacuum distillation and gas chromatography mass spectrometry for determination of low levels of volatile nitrosamines in meat products. *J. agric. Food Chem.*, **19** (5): 937–940.

TELLING G. M., BRICE, T. A., HOAR, D., OSBORNE, D., & WELTI, D. (1974) *Progress in the analysis of volatile N-nitroso compounds.* IARC Sci. Publ. No. 9, pp. 12–17.

TERRACINI, B., MAGEE, P. N., & BARNES, J. M. (1967) Hepatic pathology in rats on low dietary levels of dimethylnitrosamine. *Br. J. Cancer*, **21**: 559 565.

THOMAS, J. F. J. (1948) *Water Survey Report No. 2, Ottawa river drainage basin.* Canada Department of Mines and Technical Surveys, pp. 11–23 (Publ. No. 834).

TOMATIS, L. & CEFIS, F. (1967) The effects of multiple and single administration of dimethylnitrosamine to hamsters. *Tumori*, **53**: 447–451.

TOMATIS, L. (1977) Prenatal exposure to chemical carcinogens and its effect on subsequent generations. In: *Conference on Perinatal Carcinogenesis.* Washington, DC, National Cancer Institute Monograph.

TOMLINSON, T. E. (1970) Trends in nitrate concentrations in English rivers in relation to fertilizer. *Water Treat. Exam.*, pp. 277 293.

TOUSSAINT, W. & SELENKA, F. (1970) Methemoglobin formation in young infants. A contribution to drinking water hygiene on Rheinhessen. *Mschr. Kinderheilk.*, **118** (6): 282–284.

UNGERER, O., EISENBRAND, G., & PREUSSMANN, R. (1974) The reaction of nitrite with pesticides. Formation, chemical properties and carcinogenic activity of the *N*-nitroso compounds of the herbicide *N*-methyl-*N*¹-(2-benzothiazolyl)-urea. *Z. Krebsforsch.*, **81** (3–4): 217–242.

UNITED NATIONS (1976) Statistical Yearbook, 1975, New York, p. 298.

US DEPARTMENT OF AGRICULTURE (1965) *Agricultural Information Bulletin*, No. 299, Washington, DC.

105

US Department of Health, Education and Welfare (1966) *Air Quality Data from the National Air Sampling Networks and Contributing State and Local Networks, 1964–1965*, Cincinnati, OH, USDHEW Public Health Service Division of Air Pollution, pp. 125.

US Department of Health, Education and Welfare (1970) *Nitrates, nitrites and methaemoglobinaemia*, Research Triangle Park, N.C., National Institute of Environmental Health Sciences, pp. 43 (Environ. Rev. No. 2).

Van Logten, M. J., Den Tonkelaar, E. M., Kroes, R. Berkvens, J. M., & Van Esch, G. J. (1972) Long-term experiment with canned meat treated with sodium nitrate and glucono-δ-lactone in rats. *Food Cosmet. Toxicol.*, **10**: 475–488.

Van Rensburg, S. J. (1972) Failure of low protein and zinc intakes to influence nitrosamine-induced oesophageal carcinogenesis. *S. Afr. med. J.*, **46**: 1137-1138.

Vavra, J. J., De Boer, C., Dietz, A., Hanka, L. J., & Sokolski, W. T. (1960) *Antibiotics Annual 1959–1960*, NY, Antiobiotica Inc, pp. 230.

Verly, W. G., Barbason, H., Dusart, J., & Petitpas-Dewandre, A. (1967) A comparative study of the action of ethyl methane sulphonate and HNO_2 on the mutation to streptomycin resistance of *Escherichia coli* K 12. *Biochim. Biophys. Acta*, **145**: 752–762.

Vesselinovitch, S. D. (1969) The sex-dependent difference in the development of liver tumors in mice administered dimethylnitrosamine. *Cancer Res.*, **29**: 1024–1027.

Viets, F. B. & Aldrich, S. R. (1973) *Nitrogenous materials in the environment*, Draft Report, US Environmental Protection Agency, Hazardous Materials Advisory Committee.

Vigil, J., Warburton, S., Haynes, W. A., & Kaiser, L. R. (1965) Nitrates in municipal water supply cause methemoglobinemia in infant. *Public Health Rep.*, **80** (12): 1119 1121.

Von Kreybig, T. (1965a) [The effect of teratogenic compounds on the early stages of prenatal development in the rat.] *Naunyn-Schmiederbergs Arch. exp. Path. Pharmacol.*, **252**: 196-204 (in German).

Von Kreybig, T. (1965b) [The effect of carcinogen methylnitrosourea doses on the embryonal development in the rat.] *Z. Krebsforsch.*, **67**: 46–50 (in German).

Von Kreybig, T. (1968) [Experimental prenatal toxicology.] *Arzneimittel-Forsch.*, Suppl. 17, pp. 1 211 (in German).

Von Kreybig, T. & Schmidt, W. (1966) [On chemical teratogenesis in the rat.] *Arzneimittel-Forsch.*, **8**: 989 1001 (in German).

Von Kreybig, T. & Schmidt, W. (1967) [Chemically induced fetopathies in the rat.] *Arzneimittel-Forsch.*, **9**: 1093 1100 (in German)

Voogt. P. (1906) The determination of nitrate with a nitrate selective electrode. *Dtsch Lebensm. Rundschau.*, **65**: 196 198.

Walker, E. A., Pignatelli, B., & Castegnaro, M. (1975) Effects of gallic acid on nitrosamine formation. *Nature (Lond.)*, **258**: 176.

Walker, E. A., Bogovski, P., & Gricuite, L., ed. (1976) Environmental N-nitroso compounds analysis and formation. *Proceedings of a Working Conference, the Polytechnical Institute, Tallinin, Estonian SSR, 1–3 October, 1975*, Lyons, International Agency for Research on Cancer, pp. 512.

Walters, C. L. (1971) The detection and estimation of trace amounts of N-nitrosamines in a food matrix. *Lab. Pract.*, **20**: 574 578.

Walton, G. (1951) Survey of literature relating to infant methemoglobinemia due to nitrate-contaminated water. *Am. J. Public Health*, **41**: 986-996.

Wasserman, A. E., Fiddler, W., Doerr, R. C., Osman, S. F., & Dooley, C. J. (1972) Dimethylnitrosamine in frankfurters. *Food Cosmet. Toxicol.*, **10**: 681–684.

Water Research Centre (1974) *Notes on water pollution*, No. 66, pp. 4.

Watrous, R. M. (1947) Health hazards of the pharmaceutical industry. *Br. J. ind. Med.*, **4**: 111- 125.

Weast, R. C. ed. (1976) *Handbook of chemistry and physics*, 57th edition, Cleveland, OH, CRC Press, pp. C-81-C-685.

Weschler, W. (1970) Oncogenic, teratogenic and mutagenic effects of methyl- and

ethylnitrosourea. In: *6th International Congress of Neuropathology, Paris.* Masson, pp. 128 129.

WESCHLER, W. (1971) Teratogenic effects of the neurotropic resorptive carcinogens methyl- and ethylnitrosourea in rats. *J. Neuropath. exp. Neurol.*, **30:** 120–121.

WESCHLER, W. (1973) *Carcinogenic and teratogenic effects of ethylnitrosourea and methyl-nitrosourea during pregnancy in the rat.* IARC Sci. Publ. No. 4, pp. 127 142.

WEGNER, T. N. (1972) Simple and sensitive procedure for determining nitrate and nitrite in mixtures in biological fluids. *J. Dairy Sci.*, **55** (5): 642-644.

WEIL, L. & QUENTIN, L.-E. (1973) Nitrogen compounds in water and nitrate determination with ion-sensitive electrode. *Vom Wasser*, **40:** 125- 133.

WEISBERGER, J. H. & RAINERI, R. (1975) Dietary factors and the etiology of gastric cancer. *Cancer Res.*, **35:** 3469–3474.

WEISBERGER, E. K., WARD, J. M., & BROWN, C. (1974) Dibenamine: Selective protection against diethylnitrosamine-induced hepatic carcinogenesis but not oral, pharyngeal and esophageal carcinogenesis.*Toxicol. appl. Pharmacol.*, **28:** 477 484.

WELLS, B. E., WALKER, R., & WALTERS, C. L. (1974) Interactions between sodium nitrite and foodstuffs under gastric conditions. *J. Sci. Food Agric.*, **25:** 1048.

WHO (1971) *International standards for drinking water.* Third ed, Geneva, World Health Organization, pp. 70.

WHO (1977) *Environmental health criteria 4-- Oxides of nitrogen*, Geneva, World Health Organization, pp. 81.

WILLIAMS, A. O. (1970) Ultrastructure of liver cell carcinoma in *Macaca mulata monkey. Exp. mol. Pathol.*, **13:** 359 369.

WINTON, E. F., TARDIFF, R. G., & MCCABE, L. J. (1971) Nitrate in drinking water. *J. Am. Water Works Assoc.*, **63:** 95-98.

WORKSHOP ON GLOBAL ECOLOGY (1971) *Man in the living environment*, The Institute of Ecology Report of the workshop on global ecological problems, Chicago, IL, Field Museum of Natural History, pp. 267.

WRIGHT, A. J. & DAVIDSON, K. L. (1964) Nitrate accumulation in crops and nitrate poisoning in animals. *Advan. Agron.*, **16:** 197–247.

WRIGLEY, F. (1948) Toxic effects of nitroso-methyl urethane. *Br. J. ind. Med.*, **5:** 26–27.

YANG, K. W. & BROWN, E. V. (1972) A sensitive analysis for nitrosamines. *Anal. Lett.*, **5** (5): 293 304.

ZALDIVAR & WETTERSTRAND (1975) Further evidence of a positive correlation between exposure to nitrate fertilizers ($NaNO_3$ and KNO_3) and gastric cancer death rates: Nitrites and nitrosamines. *Experientia (Basel)*, **31:** 1354 1355.

ZIEBARTH, D. & SCHEUNIG, G. (1976) *Effects of some inhibitors on the nitrosation of drugs in human gastric juice.* IARC Sci. Publ. No. 14, pp. 279–290.

ZIMMERMAN, F. K. & SCHWAIER, R. (1967) Induction of a mitotic gene conversion with nitrous acid, 1-methyl-3-nitro-1-nitrosoguanidine and other alkylating agents in *Saccharomyces cerevisiae. Mol. gen. Genet.*, **100:** 63-76.

ZIMMERMAN, F. K., SCHWAIER, R., & LAER, U. V. (1966) Mitotic recombination induced in *Saccharomyces cerevisiae* with nitrous acid, diethylsulphate and carcinogenic, alky-lating nitrosamides. *Z. Vererbungsl.*, **98:** 230 246.