PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Nitrogen as a Potential Health Hazard

P. N. Magee

Phil. Trans. R. Soc. Lond. B 1982 **296**, 543-550
doi: 10.1098/rstb.1982.0025

Phil. Trans. R. Soc. Lond. B **296**, 543–550 (1982)
Printed in Great Britain

Nitrogen as a potential health hazard

By P. N. Magee

Fels Research Institute and Department of Pathology, Temple University School of Medicine, 3420 North Broad Street, Philadelphia, Pennsylvania 19140, U.S.A.

Possible ways in which disturbances of the nitrogen cycle might result in deleterious effects on human and animal life are discussed, including the hazardous properties of oxides of N, nitrate and nitrite, and certain *N*-nitroso compounds that may be found in the environment. The main biological actions of oxides of N are caused by NO_2, which is a powerful respiratory irritant. Nitrates have relatively low toxicity but nitrites can cause methaemoglobinaemia, which may be fatal, particularly in infants. Nitrosamines occur in the environment in very low concentrations in certain foods, in tobacco smoke, and in the atmosphere in some areas. Carcinogenic nitrosamines can also be formed in the body by reaction of endogenous amines with nitrites, part of the latter being derived from saliva. Although nitrosamines are established as powerful carcinogens in animals, their possible role in human cancer is uncertain and requires further study.

Introduction

The title of this presentation is rather misleading and could perhaps have been better expressed as: 'Some potential or possible health hazards that might arise from perturbation of the nitrogen cycle.' I have previously discussed this topic (Magee 1977) when participating in a Nobel Symposium on 'Nitrogen – an essential life factor and a growing environmental hazard' (see Bolin & Arrhenius 1977). Some potentially hazardous N compounds whose levels in the environment might be increased in this manner are gaseous oxides of N, nitrates and nitrites, nitrosamines and other *N*-nitroso compounds. Ammonia has not been included because its irritant and toxic properties are well known and a recent comprehensive review (National Research Council 1979) has concluded from the limited evidence available that concentrations of ammonia encountered in the workplace or on the farm do not present a problem in terms of a hazard to human health. Even less information is available on the effects of ammonia encountered in the urban environment on the general population.

Oxides of N occurring in the atmosphere

The main oxides of N that occur in the atmosphere are: nitrous oxide (N_2O), nitric oxide (NO), nitrogen dioxide (NO_2), nitrogen trioxide (N_2O_3), nitrogen pentoxide (N_2O_5) and nitrate ion (NO_3^-). Possible adverse effects of these pollutants on human health have been discussed by Knelson & Lee (1977) and comprehensively reviewed by the Committee on Medical and Biologic Effects of Environmental Pollutants (National Academy of Sciences 1977).

Of the oxides of N, NO and NO_2 are of major concern as possible health hazards and will be considered first. Certain organic nitrates may cause skin irritation and possibly play a role in the causation of human skin cancer. N_2O, at the levels found in the atmosphere, is probably

of no concern, but its possible effects on the stratospheric ozone layer may have implications for human health. The two last compounds will be discussed after consideration of NO and NO_2.

NO and NO_2 are interconvertible in the atmosphere and are usually designated NO_x. Since NO is rapidly oxidized to NO_2, the greater part of the published work on NO_x relates to NO_2. Acute toxic effects of high atmospheric concentrations of NO_2 have resulted from occupational exposure experienced by welders, silo fillers, miners, chemists, firemen and workers in nitric acid plants. The gas is highly irritant to the tissues of the respiratory tract after inhalation, causing varying combinations of bronchitis, bronchiolitis, pneumonitis, pneumonia and pulmonary oedema, depending on the quantity and concentrations inhaled. Short-term exposures to 300 mg NO_2 m^{-3} have been fatal while similar exposures to concentrations of 50–200 mg NO_2 m^{-3} have caused severe but reversible pulmonary effects. Clearly the higher is the air concentration the more severe are the acute reactions. The earliest response to NO_2 occurs in the sense organs. Its odour can be perceived at levels of about 0.23 mg m^{-3} (0.12 µl l^{-1}) and effects on human dark adaptation have been reported to occur at atmospheric levels between 0.14 and 0.50 mg m^{-3} (0.075–0.26 µl l^{-1}). These responses are regarded as physiological and rapidly reversible with no evidence of deleterious consequences. Studies of human airway resistance by several investigators have indicated that the earliest measurable effects occur at atmospheric concentrations of 2.8–3.8 mg m^{-3} (1.5–2.0 µl l^{-1}). These effects were again reversible in healthy subjects but were considered to be potentially adverse in asthmatic subjects or sufferers from chronic obstructive pulmonary disease. Effects of prolonged exposure to NO_2 on human pulmonary functions are difficult to assess from the limited epidemiological studies available because the populations investigated have also been exposed to other atmospheric pollutants.

An important finding from studies in experimental animals is that exposure to NO_2 reduces resistance to infection with pathogenic microorganisms that cause lung disease. This deleterious effect has been observed in mice, hamsters and squirrel monkeys infected with a range of microorganisms including *Klebsiella pneumoniae*, *Staphylococcus aureus* and influenza virus. There is some suggestion of a similar reduction in resistance to pulmonary infections in human beings living in areas of relatively high exposure to NO_2, for example in schoolchildren living at varying distances from a plant manufacturing trinitrotoluene in Chattanooga, Tennessee. Problems of interpretation of the data arose, however, in this and other studies because of concurrent exposure to sulphur dioxide and particulates.

It may be concluded that the acute irritant effects of NO_x are reasonably well understood and that deleterious results from these effects on the health of healthy human beings are unlikely to arise in most areas. The possibility that atmospheric concentrations of NO_x too low to induce detectable toxic actions may reduce resistance to pulmonary infections must be considered, but the available evidence seems to indicate that this does not constitute a serious human health hazard in most areas at present.

Atmospheric nitrates have been discussed in detail by Lovelock (1977), who suggests the possibility that peroxyacetyl nitrate (PAN) may be carcinogenic and possibly play a role in the causation of human skin cancer. Alkyl and acyl peroxynitrates are among the many products of the atmospheric oxidation of hydrocarbons associated with the photochemical smog of cities such as Los Angeles. They are currently not detectable in the troposphere, but Lovelock and others (Lovelock 1977) have shown that they can form rapidly and in easily detectable

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

concentrations on suitable surfaces exposed to the air. Lovelock points out that the chemical classes peroxides and hydroperoxides, as well as esters of strong acids, include members that are shown to be mutagenic and suspected as potential human carcinogens. He goes on to suggest that PAN and related compounds may be related to the increasing incidence of human melanoma of the skin which, he maintains, cannot be explained on the basis of exposure to sunlight alone.

N_2O, apart from its well known anaesthetic effects, is of relatively low toxicity and the concentrations occurring in the atmosphere are too low to cause concern as a human health hazard. It has been suggested, however, that increasing release of N_2O into the atmosphere from fossil fuel combustion and from its use as a propellant in various aerosol canisters may so increase the concentration in the stratosphere that it may reduce the ozone layer. Since the ozone layer plays an essential role in filtering out ultraviolet radiation from the solar spectrum, anything that diminishes this layer could reduce the effectiveness of this process and thus lead to increased human exposure. Ultraviolet radiation is established as a major causal factor in human skin cancer (National Academy of Sciences 1973) and it is thus possible that increased release of N_2O from the surface of the Earth, from whatever source, might indirectly result in an increased incidence of this form of human cancer.

Nitrates and nitrites

Some biological effects of nitrates and nitrites are shown in table 1. The toxicity of nitrates and nitrites and their environmental implications for human health have been discussed in detail in several recent reviews (National Academy of Sciences 1972, 1978; Lijinsky 1979*a*, Green *et al.* 1981).

Table 1. Nitrates and nitrites

	nitrates	*nitrites*
toxicity	relatively non-toxic	methaemoglobinaemia and other effects
mutagenicity		mutagenic in microorganisms
carcinogenicity		*in vitro*: malignant transformation of cultured cells *in vivo*: disputed reports; currently under intensive discussion

Nitrates have relatively low toxicity in animals and man, probably because they are rapidly absorbed from the stomach and the lungs (if inhaled) and rapidly excreted in the urine. Acute nitrate poisoning in man has been described after the ingestion of 8–15 g of nitrate, the clinical picture being rapid severe gastroenteritis with abdominal pain, blood in the urine and stools, weakness and collapse. Such acute poisoning is uncommon. Repeated smaller doses of nitrate result in dyspepsia, mental depression and headache of unknown mechanism. Little is known about the chronic effects of nitrates, which are reported to include vitamin A deficiency and thyroid depression in animals. It is thus clear that nitrates as such do not constitute a serious hazard but their ingestion may result in severe toxicity because of conversion in the body to nitrites. Reduction of nitrate to nitrite can occur through the action of the intestinal flora in some animals and in the stomach of the human infant during the first 3 or 4 months of life. The presence of nitrate reductase enzymes has been demonstrated in several rat tissues (Cohen & Weinhouse 1971) and there is evidence that xanthine oxidase and aldehyde oxidase can also catalyse this reduction (Rajagopalan *et al.* 1962), but the significance of these reactions for

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

nitrate toxicity is not clear. Formation of nitrite from nitrate may also occur during storage of some vegetables such as spinach.

In contrast to nitrates, nitrites are acutely toxic, mainly through induction of methaemoglobinaemia, a condition that occurs more readily in human infants than adults and to which infants are more susceptible. An excellent account of the chemistry of methaemoglobin and the biological consequences of its formation *in vivo* is provided by Green *et al.* (1981). Nitrites are rapidly absorbed from the stomach into the bloodstream where, among other reactions, they convert the iron of the haemoglobin to the ferric state to form methaemoglobin, which cannot function as an oxygen carrier. If more than 5% of the haemoglobin is converted to methaemoglobin the infant becomes cyanosed, at 30–40% conversion hypoxia sets in, and levels of methaemoglobin greater than 50% are usually fatal. There are three reasons why human infants during the first 3 months of life are unusually susceptible to methaemoglobin induction by nitrates and nitrites. At this age the pH of the stomach contents is relatively high compared with that of the adult, i.e. in the range pH 5–7. As a result of this increased pH, organisms that reduce nitrate, which are normally found only in the intestine, can colonize the stomach. Secondly, foetal haemoglobin, or haemoglobin F, which occurs during foetal life, persists during early infancy and this form of haemoglobin is more readily oxidized to methaemoglobin than adult haemoglobin. Thirdly, infant red cells have less activity of the NADH-dependent enzyme methaemoglobin reductase, which is responsible for the reduction of methaemoglobin, than adult red cells (see Jaffe (1981) for a recent review of methaemoglobinaemia).

Although there are a number of recorded cases of infant methaemoglobinaemia resulting from nitrite ingestion, it is not a common condition and has only rarely arisen from consumption of foods containing nitrites. The majority of cases in the United States and elsewhere have been caused by various infant milk formulations that have been prepared from well water containing excessive concentrations of nitrates. It is important to emphasize the association of the use of private well water with the causation of infant methaemoglobinaemia and it seems reasonable to conclude that the U.S. Public Health Service Standard of 10 mg NO_3-N l^{-1} of drinking water, or less, should be adequate protection against the condition.

The mutagenicity of nitrites in microorganisms is well established (reviewed by Zimmermann 1977). Several possible mechanisms have been proposed, including deamination of DNA bases, cross-linking of DNA and localized nitrosation reactions (Hartman 1980). There seems to be no published evidence for the mutagenicity of nitrite in mammalian cells, however, apart from one report, where the concentration of nitrite required was very high (Kodama *et al.* 1976).

The present position with regard to the possible carcinogenicity of nitrites is confused and controversial. Neoplastic transformation *in vitro* of newborn hamster cells by sodium nitrite was reported by Tsuda *et al.* (1976). Using relatively high concentrations of sodium nitrite (50 or 100 mM) these authors showed that exposure of mass cultures of newborn hamster cells resulted in morphological transformation and that two of five transformed cultures produced progressively growing tumours (fibrosarcomas) when injected into young adult hamsters. They discussed the negative results of several feeding experiments in rodents where nitrite had been fed for prolonged periods as a control for other groups receiving simultaneous treatment with the same dose of nitrite together with a nitrosatable amine, and concluded that further long-term studies with nitrite *in vivo* were required. During the course of one such study Shank & Newberne (1976) reported the effects of feeding sodium nitrite and morpholine, either singly or together, to Sprague–Dawley rats. Under these conditions sodium nitrite alone, at a dietary level of 1 g kg^{-1}

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

diet resulted in an incidence of 27% of tumours of the lympho-reticular system, compared with an incidence of about 12% in untreated rats. Subsequent work by Newberne (1979), using larger numbers of rats, led him to the conclusion that there was a statistically significant increase in the incidence of lymphoid tumours if the results from all the nitrite-treated rats were combined. These findings proved highly controversial and the histological material from all the animals in the studies of Newberne was reviewed by a panel of expert pathologists who did not, in a number of instances, confirm the diagnoses (Universities Associated for Research and Education in Pathology 1980). Taking all the evidence into consideration, it appears that sodium nitrite has not been shown conclusively to be a carcinogen in experimental animals.

Nitrosamines and other *N*-nitroso compounds

The chemical structures of some *N*-nitroso carcinogens are shown in figure 1. These compounds are cytotoxic, carcinogenic, mutagenic and teratogenic, and some of them are currently used in the chemotherapy of human cancer. The carcinogenicity of the nitrosamines has been extensively reviewed (Magee & Barnes 1967; Druckrey *et al.* 1967; Magee *et al.* 1976)

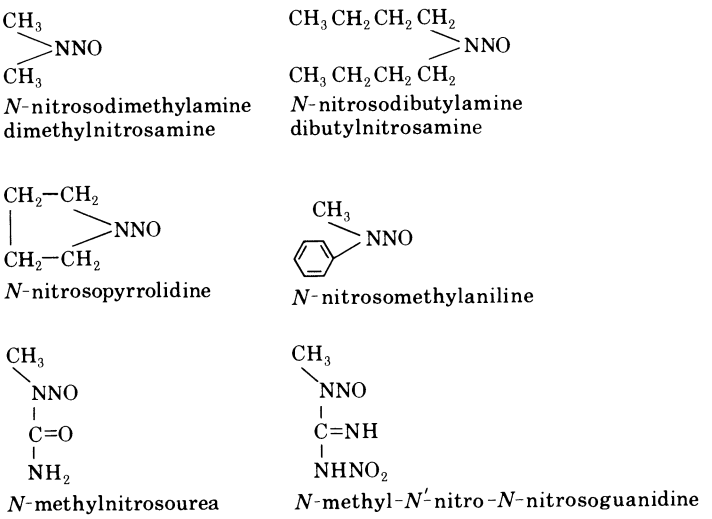

Figure 1. Some carcinogenic *N*-nitroso compounds.

and their possible role as environmental carcinogens has been discussed (Lijinsky 1979*a*). A wide range of organs in rodents and other species are susceptible to the carcinogenic nitroso compounds and there is often remarkable organ specificity that varies with the chemical structure. Although there is no direct evidence that the nitrosamines are carcinogenic in man, a recent compilation by the Soviet scientists Bogovski & Bogovski (1981) lists 39 different susceptible animal species, including monkeys and other mammals, birds, fish and reptiles. It therefore seems improbable that human beings are resistant. A recent case of homicide in West Germany, where the victim survived for $3\frac{1}{2}$ years after repeated doses of dimethylnitrosamine, provided information on chronic nitrosamine poisoning in a human female. At autopsy the liver of the victim showed severe fibrosis and nodular hyperplasia (Fussgaenger & Ditschuneit 1980), which are sometimes forerunners of primary liver cancer.

Nitrosamines and other *N*-nitroso compounds are formed by the nitrosation of a variety of

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

secondary or tertiary amines (see Magee *et al.* (1976), Mirvish (1977), Swann (1977), Lijinsky (1979*b*, 1980) and Green *et al.* (1981) for detailed discussion of the chemistry of *N*-nitroso compounds and of nitrosation reactions). In the present context the main nitrosating agent is nitrous acid, generated from nitrites under mildly acid conditions such as those occurring in the mammalian stomach.

The possibility of hazard arising from the formation of nitrosamines in food was dramatically illustrated when an outbreak of severe liver disease in sheep in Norway was found to have been caused by the formation of large amounts of dimethylnitrosamine in their diet that consisted of fish meal with nitrite added as a preservative. In this case the partly decomposed fish gave rise to large quantities of methylamines, which were nitrosated by the added nitrite (Ender *et al.* 1964). At this time sufficiently sensitive and specific analytical methods were not available for detection and determination of nitrosamines in food for human consumption. During the past decade, however, these methods have been greatly improved and it is now possible to assay nitrosamines in food and other matrices at levels in the nanograms per gram range. Several nitrosamines have been detected in various human foods, particularly those, such as bacon and ham, containing added nitrite as a preservative. The amounts of the nitrosamines that have been measured have been very small, however, and their significance for human health has been extensively but inconclusively discussed at national and international levels. However, some countries have introduced legislation requiring a reduction in the amount of nitrite and nitrate that can be deliberately added to food for human consumption on the basis of possible nitrosamine formation. As indicated above, a recent controversial report (Newberne 1979) suggested that nitrite itself may be carcinogenic. If this claim could be substantiated, there would be a clear case in the United States for banning the addition of nitrites to foods for human consumption. At present, however, the U.S. Government has not taken such action.

The formation of *N*-nitroso compounds in the animal body, after simultaneous oral administration of a variety of amines and nitrite, is well established and the actual induction of tumours in experimental animals has resulted from such treatments (Mirvish 1977; Lijinsky 1980). Since nitrite is present in human saliva in concentrations in the range 6–10 $\mu g\ g^{-1}$ (Tannenbaum *et al.* 1974) and a variety of secondary and tertiary amines may be ingested in the form of food additives, pesticide residues and normal food constituents, there is a strong probability that some nitrosation must occur in the human stomach. This has recently been clearly demonstrated to occur in a human volunteer who ingested proline together with vegetables known to contain nitrates in relatively large amounts. The identification of *N*-nitrosoproline in the urine of the volunteer showed that nitrosation of this naturally occurring imino acid did occur in a human subject and furthermore that the reaction could be inhibited by simultaneous ingestion of ascorbic acid (Ohshima & Bartsch 1981). The implications of these recent findings are of great interest because they may provide a safe and relatively simple procedure for monitoring endogenous nitrosation in different geographical areas.

Endogenous nitrosation reactions and the formation of nitrosamines *in vitro* are thus one of the reasons for the great current interest in accumulation of nitrate in the environment since massive increases in nitrate might be implicated in the formation of larger amounts of these carcinogenic substances, inside and outside the body. It should always be remembered, however, that chemical carcinogenesis is probably a multi-stage process, subject to a variety of modifying factors, and that the initiating changes in cancer induction, which probably involve interaction with and modification of DNA of target organs, may also be subject to a variety of repair processes.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Conclusions

1. Nitrates have, in themselves, relatively low toxicity and are only likely to give rise to health hazards in man or animals after reduction to nitrites.

2. Nitrites are considerably more toxic than nitrates and they have caused human illness, occasionally fatal, by the induction of methaemoglobinaemia. Young infants are particularly susceptible. There is some very controversial evidence that nitrites are carcinogenic in rats, but this is certainly not established. Under certain conditions nitrites react with various amines to give nitrosamines and other *N*-nitroso compounds.

3. Nitrosamines are powerful carcinogens and mutagens that occur in the environment and can be formed in the body from non-carcinogenic precursors. The possible role of nitrosamines in the causation of human cancer is not known and is under intensive investigation throughout the world.

Note added in proof (22 *October* 1981). The recent article by Z. M. Iqbal, K. Dahl & S. S. Epstein (Biosynthesis of dimethylnitrosamine in dimethylamine-treated mice after exposure to nitrogen dioxide; *J. natn. Cancer Inst.* **67**, 137–141 (1981)) indicates the potential carcinogenic hazard that may arise from inhalation of nitrogen dioxide.

I acknowledge support from N.I.H. Research Grants nos CA 12227 and CA 23451 from the National Cancer Institute, and from the Samuel S. Fels Fund of Philadelphia.

References (Magee)

Bogovski, P. & Bogovski, S. 1981 Animal species in which *N*-nitroso compounds induce cancer. *Int. J. Cancer* **27**, 471–474.

Bolin, B. & Arrhenius, E. (eds) 1977 Nitrogen – an essential life factor and a growing environmental hazard (Report from Nobel Symposium no. 38). *Ambio* **6**, 96–180.

Cohen, B. S. & Weinhouse, S. 1971 Reduction of nitrate to nitrite in tissues of the rat. Presented at 162nd American Chemical Society National Meeting, Washington, D.C. Abstract no. 179.

Druckrey, H., Preussmann, R., Ivankovic, S. & Schmahl, D. 1967 Organtrope carcinogene Wirkungen bei 65 verschiedenen *N*-nitroso-Verbindungen an BD-Ratten. *Z. Krebsforsch.* **69**, 103–201.

Ender, F., Havre, G., Helgebostad, A., Koppang, N., Madsen, R. & Ceh, L. 1964 Isolation and identification of a hepatotoxic factor in herring meal produced from sodium nitrite preserved herring. *Naturwissenschaften* **51**, 637.

Fussgaenger, R. D. & Ditschuneit, H. 1980 Lethal exitus of a patient with *N*-nitrosodimethylamine poisoning, 2.5 years following the first ingestion and signs of intoxication. *Oncology* **37**, 273–277.

Green, L., Ralt, D. & Tannenbaum, S. R. 1981 Nitrate, nitrite, and *N*-nitroso compounds: biochemistry, metabolism, toxicity and carcinogenicity. In *Biochemistry of nutrition II* (ed. A. Neuberger & T. H. Jukes) (*International review of biochemistry*). Baltimore: University Park Press. (In the press.)

Hartman, P. E. 1980 Bacterial mutagenesis: review and new insights. *Environ. Mutat.* **2**, 3–16.

Jaffe, E. R. 1981 Methemoglobinemia. *Clin. Hematol.* **10**, 99–102.

Knelson, J. H. & Lee, R. 1977 Oxides of nitrogen in the atmosphere: origin, fate and public health implications. *Ambio* **6**, 126–130.

Kodama, F., Umeda, M. & Tsutsui, T. 1976 Mutagenic effect of sodium nitrite on cultured mouse cells. *Mutat. Res.* **40**, 119–126.

Lijinsky, W. 1979*a* *N*-nitrosamines as environmental carcinogens. In *N-Nitrosamines* (ed. J. P. Anselme) (*A.C.S. Symp. Ser.* no. 101), pp. 165–173. Washington, D.C.: American Chemical Society.

Lijinsky, W. 1979*b* Current concepts in the toxicology of nitrates, nitrites, and nitrosamines. In *Advances in modern toxicology* (ed. M. A. Mehlman, R. E. Shapiro & H. Blumenthal), vol. 1, pp. 149–164. New York: Hemisphere Publishing Co.

Lijinsky, W. 1980 Significance of *in vivo* formation of *N*-nitroso compounds. *Oncology* **37**, 223–226.

Lovelock, J. E. 1977 PAN in the natural environment: its possible significance in the epidemiology of skin cancer. *Ambio* **6**, 131–133.

Magee, P. N. 1977 Nitrogen as health hazard. *Ambio* **6**, 123–125.
Magee, P. N. & Barnes, J. M. 1967 Carcinogenic nitroso compounds. *Adv. Cancer Res.* **10**, 163–246.
Magee, P. N., Montesano, R. & Preusmann, R. 1976 *N*-nitroso compounds and related carcinogens. In *Chemical carcinogens* (ed. C. E. Searle) (*A.C.S. Monogr.* no. 173), pp. 491–625. Washington, D.C.: American Chemical Society.
Mirvish, S. S. 1977 *N*-nitroso compounds: their chemical and *in vivo* formation and possible importance as environmental carcinogens. *J. Toxicol. Environ. Hlth* **2**, 1267–1277.
National Academy of Sciences – National Research Council 1972 *Accumulation of nitrate.* Committee on Nitrate Accumulation, National Academy of Sciences, Washington, D.C. (106 pages.)
National Academy of Sciences, Environmental Studies Board 1973 Sunlight and the common malignant tumors of the skin. In *Ad Hoc Panel on the Biological Impacts of Ultraviolet Radiation*, pp. 27–32.
National Academy of Sciences – National Research Council, Committee on Medical and Biologic Effects of Environmental Pollutants 1977 *Nitrogen oxides.* Washington, D.C.: National Academy of Sciences. (333 pages.)
National Academy of Sciences – National Research Council, Environmental Studies Board 1978 *Nitrates: an environmental assessment.* Washington, D.C.: National Academy of Sciences. (723 pages.)
National Research Council, Committee on Medical and Biologic Effects of Environmental Pollutants 1979 *Ammonia.* Baltimore, Maryland: University Park Press. (384 pages.)
Newberne, P. M. 1979 Nitrite promotes lymphoma incidence in rats. *Science, N.Y.* **204**, 1079–1081.
Ohshima, H. & Bartsch, H. 1981 Quantitative estimation of endogenous nitrosation in humans by monitoring *N*-nitrosoproline excreted in the urine. *Cancer Res.* **41**. (In the press.)
Rajagopalan, K. V., Fridovich, I. & Handler, P. 1962 Hepatic aldehyde oxidase. I. Purification and properties. *J. biol. Chem.* **237**, 922–928.
Shank, R. C. & Newberne, P. M. 1976 Dose–response study of the carcinogenicity of dietary sodium nitrite and morpholine in rats and hamsters. *Fd Cosmet. Toxicol.* **14**, 1–8.
Swann, P. F. 1977 Carcinogenic risk from nitrite, nitrate and *N*-nitrosamines in food. *Proc. R. Soc. Med.* **70**, 113–115.
Tannenbaum, S. R., Sinskey, A. J., Weisman, M. & Bishop, W. 1974 Nitrite in human saliva. Its possible relationship to nitrosamine formation. *J. natn. Cancer Inst.* **53**, 79–84.
Tsuda, H., Inui, N. & Takayama, S. 1976 *In vitro* transformation of newborn hamster cells induced by sodium nitrite. *Gann* **67**, 165–173.
Universities Associated for Research and Education in Pathology 1980 *Re-evaluation of the pathology findings of studies on nitrite and cancer.* Final Report on Contract no. 223–79–22. Report prepared for the Food and Drug Administration, Washington, D.C.
Zimmermann, F. E. 1977 Genetic effects of nitrous acid. *Mutat. Res.* **39**, 127–148.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES