Environmental Health Criteria 3

LEAD

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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, 1211 Geneva 27, 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 that information may be considered in the event of updating and re-evaluating the conclusions contained in the criteria documents.

Geneva, 29 April–5 May 1975

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ALA	δ-aminolevulinic acid
ALA-U	δ-aminolevulinic acid in urine
ALAD	porphobilinogen synthase (EC 4.2.1.24), δ-aminolevulinate de-hydratase, δ-aminolevulinic acid dehydratase
ALAS	δ-aminolevulinate synthase (EC 2.3.1.37), aminolevulinic acid synthetase
CP	coproporphyrins
CP-U	coproporphyrin in urine
CPG	coproporphyrinogen III
EDTA	ethylenediaminetetraacetic acid
FEP	free erythrocyte porphyrins
Hb	haemoglobin
LD_{50}	median lethal dose
PP	protoporphyrin IX
PBG	porphobilinogen
Pb-B	lead in blood
Pb-U	lead in urine
RBC	red blood cells
SGOT	aspartate aminotransferase (EC 2.6.1.1), serum glutamic oxaloacetic transaminase

A WHO Task Group on Environmental Health Criteria for Lead met in Geneva from 29 April to 5 May 1975. Dr B. H. Dieterich, Director, Division of Environmental Health, opened the meeting on behalf of the Director-General. The Task Group reviewed and revised the second draft criteria document and made an evaluation of the health risks from exposure to lead and its compounds.

The first and second drafts were prepared by Professor Paul B. Hammond of the Department of Environmental Health, The Kettering Laboratory, University of Cincinnati, Ohio, USA. The comments on which the second draft was based were received from the national focal points for the WHO Environmental Health Criteria Programme in Bulgaria, Czechoslovakia, Federal Republic of Germany, Greece, Japan, The Netherlands, New Zealand, Poland, Sweden, USA, and the USSR, and from the United Nations Educational, Scientific and Cultural Organization (UNESCO), Paris, from the United Nations Industrial Development Organization (UNIDO), Vienna, from the Centro Panamericano de Ingenieria Sanitaria y Ciencias del Ambiente (CEPIS) at Lima, Peru, and from the Health Protection Directorate of the Commission of the European Communities (CEC), Luxembourg. Comments were also received, at the request of the Secretariat from: Professor R. Goyer and Professor H. Warren, Canada; Professor J. Teisinger, Czechoslovakia; Dr S. Hernberg, Finland; Dr K. Cramer and Dr B. Haeger-Aronsen, Sweden; Dr D. Barltrop, Professor B. Clayton, Professor R. Lane, and Professor P. J. Lawther, United Kingdom; Dr J. J. Chisholm, Professor H. L. Margulis, and Dr G. Ter Haar, United States of America; and Dr D. Djuric and Professor K. Kostial, Yugoslavia.

Valuable comments were received on the third draft, resulting from the task group, from: Mr Joseph E. Faggan, Director of Petroleum Chemicals Research, Ethyl Corporation, Ferndale, Michigan, USA, and from Mr R. L. Stubbs, Director-General, Lead Development Association, London and Chairman, Statistical Committee, International Lead and Zinc Study Group.

The collaboration of these national institutions, international organizations, WHO collaborating centres, and individual experts is gratefully acknowledged. Without their assistance this document would not have been completed. The Secretariat wishes to thank, in particular, Professor Hammond for his continued help in all phases of the preparation of the document, and Dr H. Nordman of the Institute of Occupational Health,

Helsinki, who assisted the Secretariat in the final scientific editing of the document.

This document is based primarily on original publications listed in the reference section. However, several recent publications broadly reviewing health aspects of lead and its compounds have also been used. These include publications by Kehoe (1961), NAS-NRC (1972), NRC-Canada (1973), Goyer & Rhyne (1973), WHO Working Group (1973), Inter-Department Working Group on Heavy Metals (1974), SCEP (1974), Nordberg, ed. (1976). In addition, the document draws on comprehensive and useful data from the proceedings of several symposia and meetings, e.g. the "International Symposium on Environmental Aspects of Lead", Amsterdam, 1972, arranged by the Commission of the European Communities and the US Environmental Protection Agency; the "International Symposium on Recent Advances in the Assessment of the Health Effects of Environmental Pollution", Paris, 1974, jointly organized by the Commission of the European Communities, US Environmental Protection Agency, and the World Health Organization; the University of Missouri's Annual Conferences on Trace Substances in Environmental Health, Columbia, Missouri, 1967–1975; and the "International Symposium on Environmental Lead Research", Dubrovnik, 1975, organized by the Institute for Medical Research and Occupational Health, under the auspices of the Yugoslav Academy of Sciences and Arts.

Details of the WHO Environmental Health Criteria Programme, including some of the terms frequently used in the documents, may be found in the introduction to the publication "Environmental Health Criteria 1 — Mercury", published by the World Health Organization, Geneva, in 1976.

1. SUMMARY AND RECOMMENDATIONS FOR FURTHER RESEARCH

1.1 Summary

1.1.1 Analytical problems

The procurement of environmental and biological samples requires careful consideration of the special problems relating to the particular material to be analysed. In air sampling, it is most important to ensure that the sampler is placed at the breathing zone of the population group under study. For all sampling procedures and particularly for blood, external contamination is a major problem.

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Exposure to nitrogen dioxide increased the susceptibility of experimental animals to both bacterial and viral respiratory infections; this response was clearly dose-related. Results indicated that concentration had a much greater influence on the toxicity of nitrogen dioxide than length of exposure, i.e. equal concentration-time products at different exposure times were not equally hazardous.

When mice were exposed to 940 $\mu g/m^3$ (0.5 ppm) for 90 days and then artificially infected, a significant increase in the mortality rate was observed. A similar response was noted in squirrel monkeys exposed to much higher concentrations of 9400–19 000 $\mu g/m^3$ (5–10 ppm) for 1 or 2 months. When mortality rates due to respiratory infection were compared after continuous and intermittent exposure to nitrogen dioxide, there was a significant increase in both treatments with increasing length of exposure. However, for each given length of exposure, there was no statistical difference between the continuous and the intermittent exposure groups.

Nitrogen dioxide interferes with the lung's ability to remove inhaled deposited particles efficiently by altering the phagocytic, enzymatic, and functional processes of the alveolar macrophages and of the ciliated epithelial cells.

1.1.5   Effects on man

1.1.5.1   *Controlled exposures*

Studies on exposure to nitrogen dioxide in man have been conducted to determine the lowest levels at which odour can be detected and at which dark adaptation is altered. For odour perception the lowest nitrogen dioxide level was approximately 200 $\mu g/m^3$ (0.11 ppm). The lowest level for impairment of dark adaptation was reported to be 140 $\mu g/m^3$ (0.074 ppm).

Exposure to nitrogen dioxide levels of 1300–3800 $\mu g/m^3$ (0.7–2.0 ppm) for 10 min gave rise to an increase in inspiratory and expiratory flow resistance. In another study, inhalation of nitrogen dioxide concentrations of 3000–3800 $\mu g/m^3$ (1.6–2.0 ppm) for 15 min caused a significant increase in total airway resistance, which became more pronounced at concentrations above 3800 $\mu g/m^3$ (2.0 ppm). A number of authors have reported that exposure to 7500 9400 $\mu g/m^3$ (4–5 ppm) produced an increase in airway resistance and a decrease in the arterial partial pressure of oxygen and carbon monoxide diffusion capacity. However, prolongation of the exposure time to 60 min did not enhance the effect further. A recent report showed that in 13 out of 20 asthmatic subjects, the reaction to the inhalation challenge with a broncho-

constrictor (carbachol) increased significantly after exposure to a nitrogen dioxide level of 190 $\mu g/m^3$ (0.1 ppm) for 1h. Similar results were reported in a study in which healthy subjects were exposed to a combination of nitrogen dioxide at 100 $\mu g/m^3$ (0.05 ppm), ozone at 50 $\mu g/m^3$ (0.025 ppm), and sulfur dioxide at 260 $\mu g/m^3$ (0.10 ppm) for a period of 2h.

1.1.5.2 *Accidental and industrial exposures*

Exposure to high concentrations of oxides of nitrogen has been reported in various occupations.

Farmers who were exposed to silo gases from the fermentation of harvested crops were acutely affected by oxides of nitrogen, some of them fatally. It has been estimated that exposure to nitrogen dioxide levels of 560–940 mg/m^3 (300–500 ppm) may result in fatal pulmonary oedema or asphyxia and that levels of 47–140 mg/m^3 (25–75 ppm) can cause bronchitis or pneumonia.

Miners who used explosives repeatedly in their work were reported to develop chronic respiratory diseases. Analysis of the products of explosion showed the presence of oxides of nitrogen at concentrations of 88–167 ppm.

A study on surviving victims who had been exposed to the fumes of burning nitrocellulose did not reveal any differences in survival between the exposed groups and the unexposed controls over the following 30 years. Unfortunately quantitative exposure data for the various groups were not available in this study.

There are very few studies on the acute or chronic effects of low-level industrial exposures.

1.1.5.3 *Community exposures*

Several studies have been reported in which an attempt has been made to relate pulmonary function to nitrogen dioxide exposure. However, the results of all these studies have either failed to demonstrate a significant difference in lung function between the groups exposed to different levels of nitrogen dioxide, or have been confounded by the fact that relatively high concentrations of other pollutants were present.

This also applies to studies conducted to correlate the frequency of acute respiratory disease and chronic respiratory illness with concentrations of nitrogen dioxide.

For example, a study to evaluate the effects of nitrogen dioxide on the incidence of acute respiratory disease in children and their parents living

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near a large point source of this pollutant demonstrated an excess rate of respiratory illness in comparison with a control group. However, the probable contribution of other pollutants such as sulfuric acid aerosols, nitric acid fumes, and suspended nitrates, made it difficult to attribute this excess to the presence of nitrogen dioxide.

Similarly, because of relatively high exposure to other air pollutants, it has not been possible to associate observed increases in the frequency of chronic respiratory illness with a measured level of nitrogen dioxide. It has been noted, however, that these epidemiological studies seem to confirm the results of controlled studies on man and experimental animal studies.

1.1.6   Evaluation of health risks

As it has not yet been shown that the concentrations in ambient air of oxides of nitrogen, other than nitrogen dioxide, have any significant biological activity, a guideline for the protection of public health has been developed only for nitrogen dioxide.

Compared with experimental toxicological studies, there are very few epidemiological studies on the effects of either occupational or community exposures which can provide sufficient information for the assessment of health risks due to exposure to nitrogen dioxide. Thus, a health protection guideline has been developed based on data from controlled human studies and animal experiments. As previously stated, available epidemiological data tend to support these results.

A nitrogen dioxide concentration of 940 $\mu g/m^3$ (0.5 ppm) has been selected as an estimate of the lowest level at which adverse health effects due to short-term exposure to nitrogen dioxide can be expected to occur. Although the Task Group was aware that one study in man showed effects at a lower concentration, it was of the opinion that this required confirmation.

By adopting a minimum safety factor of 3–5, the Task Group agreed that a maximum one hour exposure of 190–320 $\mu g/m^3$ (0.10 0.17 ppm) should be consistent with the protection of public health and that this exposure should not be exceeded more than once per month.

A caution has been added that it might be prudent to lower this exposure limit in view of biological evidence of the interaction of nitrogen dioxide with other air pollutants present and also in view of the fact that some populations are highly sensitive to this substance.

Owing to lack of information on the effects of long-term exposure to nitrogen dioxide in man, only a short-term exposure limit has been suggested.

1.2 Recommendations for Further Research

In discussing the health risks of nitrogen dioxide exposure, the Task Group concentrated on the biological activity of nitrogen dioxide alone, rather than in conjunction with other compounds with which it is commonly associated in the ambient atmosphere. However, the Task Group was particularly concerned with the potential for enhanced biological effects in ambient situations in which peak concentrations of nitrogen dioxide and photochemical oxidants occur together. The Task Group also expressed concern over atmospheric oxidation products of nitrogen dioxide, such as nitrous and nitric acid and various nitrate compounds. Taking into consideration the biological data on the combined effects of nitrogen dioxide and oxidants, and of the nitrates in the ambient air, the Task Group made the following recommendations for research on the health effects of these substances:

a) Controlled studies on man and experimental studies on animals should be conducted to compare the reaction of sensitive biological systems at typical peak concentrations of nitrogen dioxide and ozone alone, and in combination. In animals, the infectious disease model appears to be particularly appropriate for these studies. In man, the effects should be studied of nitrogen dioxide and oxidants, alone or in combination, on airway resistance before and after administration of bronchoconstrictors.

b) Similar experimental animal and controlled human studies should be conducted to evaluate the biological effects of nitric acid and nitrates at concentrations found in ambient air.

c) The possibility of delayed effects from exposure to nitrogen dioxide and its oxidation products should be considered. These possibilities may be pursued by means of epidemiological studies and recently developed experimental techniques to assess carcinogenicity and mutagenicity.

d) While the Task Group is aware that epidemiological studies alone cannot provide a quantitative basis for evaluating the health risks of exposure to nitrogen dioxide, the importance of epidemiological studies of occupational and community groups should not be minimized. There is a particular need for long-term follow-up studies which may identify chronic or delayed and often subtle effects in cohorts of exposed populations.

e) Studies of highly sensitive subjects should be given careful consideration. Asthmatic subjects and persons with cardio-pulmonary disease should be studied with respect to functional and symptomatic changes associated with variations in the average

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hourly concentrations of nitrogen dioxide, nitrates, and related compounds. Controlled exposure of asthmatic subjects and other highly sensitive persons to nitrogen dioxide and nitrates at concentrations found in the ambient air may be undertaken, with their consent and with due consideration for the protection of subjects so exposed. It would be highly desirable to study animal models of human asthma and hypersensitivity.

2. CHEMISTRY AND ANALYTICAL METHODS

2.1 Chemical and Physical Properties

Oxides of nitrogen are usually classified in terms of the oxidation state of nitrogen (Table 1).

Table 1. Oxides of nitrogen

Name	Chemical formula
nitrous oxide	N_2O
nitric oxide	NO
dinitrogen trioxide	N_2O_3
nitrogen dioxide	NO_2
dinitrogen tetroxide	N_2O_4
dinitrogen pentoxide	N_2O_5

Nitrous oxide (dinitrogen oxide) is the most prevalent oxide of nitrogen in the atmosphere. This compound is generated by anaerobic processes in the soil and in the surface layers of the oceans and is present in the atmosphere in concentrations of about 450 $\mu g/m^3$ (0.25 ppm) (Robinson & Robbins, 1972). Although this species may play an important role in stratospheric chemistry, it is of little importance in the lower atmosphere and has no direct significance for human health.

Nitric oxide (nitrogen oxide) and nitrogen dioxide, the most abundant man-made oxides of nitrogen in urban areas, are derived from air used in high temperature combustion processes. Both nitric oxide and nitrogen dioxide are found in combustion gases but nitric oxide predominates because its formation is favoured by high temperatures. The formation of nitric oxide can be described by the following reactions (Spedding, 1974):

$$O + N_2 \rightleftarrows NO + N \tag{1}$$

$$O_2 + N \rightleftarrows NO + O \tag{2}$$

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Atomic oxygen needed in reaction (1) is produced in the flame by 2 parallel reactions:

$$CO + OH \rightleftharpoons CO_2 + H \qquad (3)$$

$$H + O_2 \rightleftharpoons OH + O \qquad (4)$$
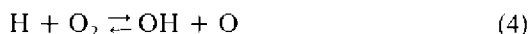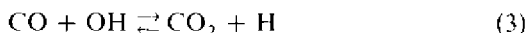
The amount of nitric oxide formed depends on the temperature of the flame, the concentrations of nitrogen and oxygen, and the residence time of gases in different zones of temperature, pressure, and concentration. Temperature is the most significant variable in the production of nitric oxide under normal combustion conditions (McKinnon, 1974). The production of nitric oxide per unit mass of fuel burned decreases with decreasing mean combustion temperature of different fuels, i.e.: coal, oil, natural gas. Because the internal combustion engine operates at a high temperature, motor vehicles are an important source of nitric oxide.

Some physical properties of nitric oxide and nitrogen dioxide are given in Table 2. Nitric oxide is a colourless, odourless gas that is slightly soluble in water. Although the boiling point of nitrogen dioxide is 21.2°C, it only exists in the gaseous form at normal air temperatures because of its low partial pressure.

Table 2. Physical properties of nitric oxide and nitrogen dioxide [a]

Oxides of nitrogen	Molecular weight	Melting point °C	Boiling point °C	Solubility in water ml/litre
nitric oxide	30.01	- 163.6	− 151.8	73.40
nitrogen dioxide	46.01	- 11.20	21.2	—

[a] From: Weast (1976)

Nitrogen dioxide is in equilibrium with its dimer, dinitrogen tetroxide ($2NO_2 \rightleftharpoons N_2O_4$) but at atmospheric concentrations the fraction of nitrogen dioxide present in dimer form is negligible.

Dinitrogen trioxide can be formed from nitric oxide and/or nitrogen dioxide. However, at the low concentrations of nitric oxide and nitrogen dioxide found even in very heavily polluted air, the chemical equilibrium data predict negligible concentrations of dinitrogen trioxide which thus has no significance as an air pollutant (Leighton, 1961).

Dinitrogen pentoxide is thought to be an important reactive intermediate in photochemical air polution, formed mainly by the oxidation of nitrogen dioxide with ozone (Demerjian et al., 1974). However, there are no specific analytical methods for the measurement of this species in ambient air and there is no evidence that it has any significance for human health.

2.2 Atmospheric Chemistry

The atmospheric chemistry of the oxides of nitrogen is very complex, particularly when other air pollutants such as hydrocarbons are present. For this reason, only a simplified account can be presented here.

Nitric oxide is fairly reactive and is readily oxidized in the atmosphere to nitrogen dioxide. The conversion takes place by means of several reactions depending on the concentrations of nitric oxide. At high concentrations, as much as 10% of nitric oxide can be oxidized by the reaction:

$$2NO + O_2 \rightarrow 2NO_2 \tag{5}$$

The rate of this reaction decreases with dilution and rapidly becomes insignificant. At low concentrations, an important reaction leading to nitrogen dioxide formation is:

$$NO + O_3 \rightarrow NO_2 + O_2 \tag{6}$$
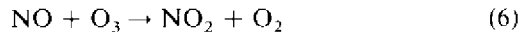
Nitrogen dioxide absorbs strongly in the ultraviolet region between 300 and 400 μm and is decomposed by sunlight yielding nitric oxide and ozone (Leighton, 1961). Thus, in daylight, reaction (6) proceeds in the opposite direction and eventually an equilibrium

$$NO + O_3 \rightleftharpoons NO_2 + O_2 \tag{7}$$
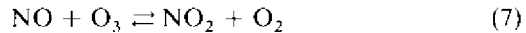
is established.

The position of equilibrium (7) is a function of the rate of reaction (6) and the rate of light absorption by nitrogen dioxide which varies with the time of the day, latitude, and other atmospheric variables (Calvert, 1976). Generally, however, in unpolluted and rural areas, the daytime concentrations of nitric oxide are only a small fraction of the concentration of nitrogen dioxide.

The air is more polluted in urban areas than in rural areas and the concentration of oxides of nitrogen is markedly higher. Thus, it is possible, particularly at night time, that reaction (6) proceeds to completion and that all ozone is removed leaving substantial concentrations of both nitric oxide and nitrogen dioxide in the atmosphere. During the day, the equilibrium (7) shifts in favour of ozone formation.

Thus, in polluted air, the position of equilibrium (7) and the resulting nitric oxide, nitrogen dioxide, and ozone concentrations depend on a large number of meteorological and other factors, and particularly on the simultaneous presence of hydrocarbon pollutants.

The pattern of nitrogen dioxide concentrations in urban air is therefore quite different from that of primary pollutants such as nitric

oxide, carbon monoxide, and sulfur dioxide. It is much more similar to that of typical secondary pollutants such as ozone and photochemical aerosol species. The differences in behaviour between nitrogen dioxide and other primary pollutants show in the relationship between peak values and long-term mean values, and in diurnal and seasonal variability. These aspects are illustrated in section 4.

The main atmospheric sink for oxides of nitrogen appears to involve its oxidation to nitric acid. This is an example of a general reaction in atmospheric chemistry where pollutants are oxidized to species which are more readily removed from the atmospheric circulation. This is particularly true for the oxides of nitrogen since nitric acid is much more soluble in water and much more readily adsorbed on the surface of suspended particulate matter.

In view of the possible effects on human health of nitrate particles, this atmospheric conversion may have an added significance. The mechanism of the conversion most probably involves hydroxyl radicals as shown by the equation (8)

$$OH + NO_2 \rightleftarrows HNO_3 \qquad (8)$$
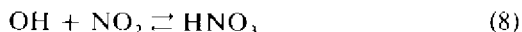
2.3 Analytical Methods

2.3.1 Sampling

Although nitric oxide and nitrogen dioxide are chemically reactive they behave quite predictably in glass and teflon sampling moulds. Residence time in the sampling manifold requires specific consideration, when sampling air containing nitric oxide, nitrogen dioxide, and ozone during daylight. Since the equilibrium (7) is disturbed inside the dark sampling manifold, nitrogen dioxide concentrations may be overestimated when sampling takes much over 10 s (Butcher & Ruff, 1971).

Collectors based on solid adsorbents that have been developed for the selective sampling of nitric oxide and nitrogen dioxide have great potential because of their stability and simplicity and because a wide selection of methods can be used for subsequent analysis. Nitrogen dioxide can be quantitatively absorbed on columns packed with an inert material coated with triethanolamine without affecting the nitric oxide concentrations (Levaggi et al., 1972). The nitric oxide is subsequently adsorbed on a second column treated with cobalt (II) oxide.

2.3.2 Evaluation of analytical methods

Detailed description of the various methods has been omitted since

they are discussed elsewhere (US Department of Health, Education and Welfare, 1965; US Environmental Protection Agency, 1971a; World Health Organization, 1976). Instead, a critical evaluation is given of the important methods used for measurements in the ambient air and in the health-effects studies discussed in subsequent sections.

Nitric oxide and nitrogen dioxide may be measured separately or collectively by manual or automated techniques.

The colorimetric manual methods are based on a specific reaction in which nitrite ions and diazotizing reagents produce a deeply coloured azo-dye (Mulik et al., 1974; Saltzman, 1954; US Environmental Protection Agency, 1971b). These methods can be automated to give mean concentrations over averaging periods of 15-60 min (Japanese Standards Association, 1974; Lyshkow, 1965; Saltzman, 1960).

The chemiluminescence techniques are automatic and specific and have revolutionized the measurement of oxides of nitrogen. The method is based on the measurement of red light produced by the reaction $O_3 + NO \rightarrow O_2 + NO_2 + light$. The major features are selective response to nitric oxide, sensitivity into the 1 $\mu g/m^3$ range, linearity over a factor of 10^5 in concentration, and rapid (< 1 s) time response (Fontijn et al., 1970; Stedman et al., 1972). High cost, complexity, and the requirement of some form of data logging system if long-term mean values are required for averaging periods from 1 day to 1 year are some drawbacks of these techniques. They are ideally suited for the measurement of peak concentrations over averaging periods of from 15 s to 1 h which are largely inaccessible with manual methods.

Besides the two basic methods there are a large number of other methods for the measurement of nitric oxide and nitrogen dioxide. Gas chromatography, long-path infrared spectroscopy, and electrochemistry have been used but either they are generally cumbersome or they have comparatively low detection limits for use in atmospheric measurements (World Health Organization, 1976).

2.3.2.1 *Manual methods*

The most widely used manual methods for nitrogen dioxide are the Saltzman method and the Jacobs-Hochheiser method; detailed analytical procedures are described elsewhere (World Health Organization, 1976). Nitric oxide can also be measured by these methods if oxidized to nitrogen dioxide prior to analysis. Although various solid and liquid oxidizing agents have been proposed for the conversion of nitric oxide to nitrogen dioxide, they are not very reliable and tend to underestimate ambient nitric oxide concentrations (World Health Organization, 1976).

Colorimetric procedures are complicated because of the time required

for the colour to develop. This colour is not permanent and the Saltzman procedure, in particular, is not therefore recommended where the samples cannot be analysed after a short time delay. The Jacobs-Hochheiser method is a useful modification of the diazotization method, applicable to 24 h samples, which can be analysed up to 1 month after collection (Jacobs & Hochheiser, 1958). However, this method has several deficiencies including a variable collection efficiency for nitrogen dioxide (Hauser & Shy, 1972).

Various modifications of the Jacobs-Hochheiser method are being evaluated. One of these, the arsenite method (Christie et al., 1970) suffers from serious interference by nitric oxide (Merryman et al., 1973). The TGS-ANSA[a] method (Mulik et al., 1974) appears to eliminate all the deficiencies of the Jacobs-Hochheiser method.

The Saltzman procedure has been used extensively in Europe, Japan, and the USA and has been tested by many workers. The method requires simple and inexpensive apparatus and its detection limit is adequate for most pollution studies. However, in view of the problems associated with the oxidation of nitric oxide to nitrogen dioxide, it is only suitable for measuring nitrogen dioxide. If nitrogen dioxide has to be monitored over periods of more than 2 h, or if the presence of relatively high concentrations of other oxidizing or reducing agents is suspected, a series of short-term samples (15–30 min each) should be collected and analysed as soon as possible. This may require some form of automatic sampling scheme.

2.3.2.2 *Automatic methods*

Continuous analysers based on the Saltzman procedure have been used extensively, but this should not be encouraged, since they are quite complicated, requiring excessive operator attention (World Health Organization, 1976). In addition, certain commercial versions suffer from interference by ozone (Baumgardner et al., 1975). Furthermore, since none of the proposed oxidizing agents for the nitric oxide-nitrogen dioxide conversion is wholly satisfactory under field conditions, continuous analysers based on the Saltzman procedure are suitable only for the measurement of nitrogen dioxide concentrations.

Chemiluminescence methods are ideally suited to the measurement of nitric oxide concentrations and are accurate and reproducible over a

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[a] TGS · absorbing solution consisting of 20 g triethanolamine + 0.5 g guaicol + 0.25 g sodium metabisulfite/litre of distilled water.
   ANSA reagent consisting of 0.1% 8-anilino-1-naphthalenesulfonic acid in absolute methanol.

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wide range of concentrations. There are no important sources of interference.

Most commercial chemiluminescence analysers for nitric oxide are also equipped with some form of converter which reduces the nitrogen dioxide to nitric oxide before reaction with ozone to yield a combined measurement of nitric oxide and nitrogen dioxide. Considerable problems may occur in the mechanics of the subtraction of the nitric oxide signal from the combined nitric oxide-nitrogen dioxide signal when the nitric oxide concentrations are much higher than the nitrogen dioxide concentrations.

Although the chemiluminescence determination of nitric oxide is interference-free, this is not always the case with the measurement of nitrogen dioxide. The conversion of atmospheric ammonia to nitric oxide can be eliminated by the appropriate choice of converter material and operating temperature. Ammonia derived from animal waste products may interfere with the determination of nitrogen dioxide exposures in the animal experiments described later. Certain nitrogen species such as nitric acid and peroxyacetylnitrate (PAN) decompose in most commercial thermal converters (Winer et al., 1974). This contributes minor interference during photochemical air pollution episodes.

2.3.3 Calibration procedures

There are 3 independent procedures for calibrating methods for measuring oxides of nitrogen. One technique involves the use of permeation tubes for nitrogen dioxide (Lindqvist & Lanting, 1972). Another technique is based on the gas-phase titration of nitric oxide with ozone which provides simultaneous calibration for nitric oxide, nitrogen dioxide, and ozone using the reaction:

$$NO + O_3 \rightarrow NO_2 + O_2 \qquad (6)$$
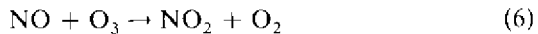
Finally, dynamic dilution can be used to prepare flowing mixtures of nitric oxide and nitrogen dioxide in air for calibration purposes (Japanese Standards Association, 1976).

There has been much discussion in the literature concerning the "Saltzman factor" i.e. the conversion factor for sodium nitrite to nitrogen dioxide. This problem arises when the method is calibrated against standard solutions of nitrite ions, and can be obviated by the calibration methods discussed above. However, it must be borne in mind when interpreting literature data where this form of calibration has been used and a value assumed for the "Saltzman factor". This factor is

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usually about 0.72 (Forweg, 1975), but may vary with experimental conditions and concentration.

3. SOURCES OF OXIDES OF NITROGEN

3.1 Natural Sources

Nitric oxide and nitrogen dioxide present in the air are produced by natural processes including lightning, volcanic eruptions, and bacterial action in the soil, as well as by man-made activities. It has been estimated that the annual, natural global emissions of these oxides of nitrogen are of the order of 1100 million tonnes (Robinson & Robbins, 1972). This by far surpasses emissions of oxides of nitrogen generated by man-made activities which were estimated in 1970 to be approximately 53 million tonnes. However, since natural emissions are distributed over the entire globe, the resulting air concentrations are practically negligible.

3.2 Man-made Sources

The major source of man-made emissions of oxides of nitrogen is the combustion of fossil fuels. The predominant oxide of nitrogen emitted by combustion processes is nitric oxide; nitrogen dioxide is produced in much smaller amounts. The observed percentage of nitric oxide in the total emission of oxides of nitrogen is 90 95% by volume although it depends on a number of factors and varies substantially from one source to another.

The distribution of emissions from different sources, in selected countries is shown in Table 3. Because emissions of oxides of nitrogen are extremely variable, these estimates provide only a general guide on the nature and magnitude of the more important sources. Generally, the differences between the various countries illustrated in Table 3 can be readily accounted for by differences in fuel use. For example, in the Netherlands a significant fraction of the electricity generating plants use natural gas whereas this use of natural gas is negligible in the UK. This greater reliance on natural gas in the Netherlands may account for the much smaller relative contribution from stationary sources. Table 3 also indicates that transportation sources are relatively more significant in Japan and the USA than in the Netherlands or the UK.

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Table 3. Emissions of oxides of nitrogen from various sources in selected countries expressed as 10^6 tonnes per year

Source	Japan[a] 1972	Netherlands[b] 1972	UK[c] 1970	USA[d] 1970
Transportation	0.96	0.13	0.45	11.7
Fuel combustion in stationary sources	1.44	0.19	1.98	10.0
Non-combustion industrial processes		—	—	0.2
Miscellaneous	—	·	—	0.9
Total	2.40	0.32	2.43	22.8

From: [a]Central Council for Environmental Pollution Control (1977).
[b]National Air Pollution Council, the Netherlands (1976).
[c]Derwent & Stewart (1973).
[d]US Environmental Protection Agency (1973).

Projection of emission data into the future must be treated with some caution. It is evident, however, that in the absence of any abatement strategies, oxides of nitrogen emissions in most urban areas will increase steadily within the next decade (Organization for Economic Cooperation and Development, 1973). Prior to the energy crisis in the 1970s, oxides of nitrogen emissions had been expected to about double between 1968 and 1980. Such projections may require reevaluation in the light of changing patterns of fuel use.

3.2.1 Stationary sources

As shown in Table 3, stationary combustion sources in Japan, the Netherlands and the UK account for 60, 59, and 82% of total emissions, respectively. In the USA this figure is about 44%. These emissions include a substantial contribution from power generating plants. In the UK and the USA, these large power plants contribute 52% and 21%, respectively, of the total emissions (Derwent & Stewart, 1973; Mason et al., unpublished data[a]).

The combustion of fuel in the home makes only a minor contribution to total emissions of oxides of nitrogen. In the UK and the USA domestic fuel use accounts for only 5% and 6% of total emissions respectively.

Fuel combustion by the commercial and industrial sectors provides a substantial source of oxides of nitrogen emissions in certain urban areas, particularly through space heating during the winter season.

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[a] Paper presented at the Sixty-second Annual Meeting, Air Pollution Control Association, June 1969, Paper No. 96 101, p. 19.

3.2.2 Mobile sources

Transportation sources include personal motor vehicles, buses, trucks, railroad vehicles, aircraft, and ships on inland waterways. Of these many categories, petrol-powered motor vehicles provide by far the largest contribution to total emissions. As a whole, transportation sources make a substantial contribution to the total emissions of the countries listed in Table 3. For Japan, the Netherlands, UK, and USA, transportation sources account for 40, 41, 18, and 51% respectively, of total emissions.

3.2.3 Non-combustion sources

Although the total emissions from industrial processes (other than from fuel combustion) are relatively small, certain processes are significant local sources of oxides of nitrogen. Examples of these non-combustion sources include the manufacture of nitric acid, electro-plating, and processes involving concentrated nitric acid such as the manufacture of explosives and the manufacture of sulfuric acid by the chamber process. The manufacture of nitric acid is usually the most significant of these non-combustion sources (Bagg, 1971).

Bacterial degradation of silage material can be a significant source of oxides of nitrogen and has led to certain occupational hazards which are mentioned in section 6.

3.2.4 Other sources

Exposure to oxides of nitrogen from home appliances such as gas stoves and from tobacco smoking should not be underestimated. Exposure levels due to these sources are discussed in section 4.

4. ENVIRONMENTAL LEVELS AND EXPOSURES

4.1 Background Concentrations

Available data indicate that natural background levels of nitrogen dioxide over land areas range from 0.4 to 9.4 $\mu g/m^3$ (0.0002-0.005 ppm) and those of nitric oxide from 0 to 7.4 $\mu g/m^3$ (0–0.006 ppm) (Robinson & Robbins, 1972). In Panama, for example, Lodge & Pate (1966) found average nitrogen dioxide values ranging from 1.7 $\mu g/m^3$ (0.0009 ppm)

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during the dry season to 6.8 μg/m³ (0.0036 ppm) in the rainy season. The natural background level of nitrogen dioxide in remote areas of Western Europe ranged from below 2.0 to 4.2 μg/m³ (below 0.0011 to 0.0022 ppm) (Georgii & Weber, 1962). These concentrations are 1-2 orders of magnitude lower than those typically found in urban areas.

4.2 Urban Concentrations

Urban concentrations of nitric oxide, nitrogen dioxide, and oxides of nitrogen have been measured in a number of countries in recent years. The results have usually been reported as 1 h, 24 h, or annual averages. Annual average nitric oxide levels in large cities have been reported to range from 49 to 95 μg/m³ (0.040–0.077 ppm) (Environment Agency, 1974; US Environmental Protection Agency, 1971a). An indication of long term average concentrations of nitrogen dioxide can be obtained from Table 4 where the annual mean concentrations are shown for selected urban areas. It is important to recognize that these concentrations did not necessarily represent the maximum exposure levels in these cities, since nitrogen dioxide concentrations vary greatly within a given urban area. It should also be remembered that different measurement methods were used in different countries and that these might have changed during the years tabulated.

Table 4. Annual mean concentrations of nitrogen dioxide in selected cities (μg/m³) [a]

Year	Rotterdam	Washington, DC	Frankfurt/ Main	Tokyo
1962		56	19	
1963		56	23	
1964		75	28	
1965		56	30	
1966	35		47	
1967	43	75	34	
1968	43		41	
1969	43		63	77
1970	45	94	82	73
1971		75	80	58

[a]From: Commissie Bodem, Water en Lucht, 1970; Environment Agency, 1976; Jost & Rudolph, 1975; US Environmental Protection Agency 1962–1971.

Thus, additional information and interpretation are required before comparisons between cities or determination of trends can be made.

Tables 5 and 6 illustrate the observed short-term mean concentrations of nitrogen dioxide. In addition to annual means, Table 5 presents the maximum 1-h, 24-h, and monthly mean concentrations of nitrogen dioxide recorded at selected sites in 5 cities in Japan (Environment Agency, 1974). Data on maximum 24-h concentrations and annual

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Table 5. Nitrogen dioxide concentrations ($\mu g/m^3$) recorded in selected cities in Japan during 1973 using the Saltzman method[a]

City[b]	Annual mean	Maximum 1-h value	Maximum 24-h mean	Maximum monthly mean
Sendai	46	240	134	60
Tokyo	86	840	426	105
Kawasaki	90	440	200	113
Osaka	86	640	228	115
Matsue	10	60	22	14

[a]From: Environment Agency, 1974.
[b]Densely populated, except Matsue.

Table 6. Annual mean and maximum 24-h nitrogen dioxide concentrations recorded in selected cities in the USA during 1974 using the chemiluminescence method[a]

City	Annual mean	Maximum 24-h mean
San Jose	67	285
Philadelphia	73	166
Washington, DC	68	130
New York	80	243
Chicago	47	114

[a]From: US Environmental Protection Agency (1976a)

means for 5 US cities are given in Table 6 (US Environmental Protection Agency, 1976a). The maximum 24-h mean concentrations of nitrogen dioxide were generally within the range of 100–400 $\mu g/m^3$ (0.05–0.22 ppm) and the maximum 1-h concentrations over 800 $\mu g/m^3$ (0.43 ppm). The maximum 24-h mean value refers to the day of the year with the highest mean concentration. The maximum 1-h value refers to the highest 1-h value in the year and the maximum monthly to the highest monthly mean in the year.

Since most air pollutant concentrations are approximately log-normally distributed, a fairly consistent relationship can be established between annual averages and the averages calculated for shorter averaging periods (Larsen, 1969). For nitrogen dioxide, the maximum 24-h mean is about 2–5 times higher than the annual mean at a given site. The relationship of the maximum 1-h value to the annual mean is not as consistent. Available data show that the hourly maximum value is approximately 5–10 times the annual mean. This relationship does not hold for averaging periods of less than 1 h or for unusual situations. The model also appears to overpredict maximum monthly mean nitrogen dioxide concentrations. Examples of the seasonal variation in nitric oxide and nitrogen dioxide concentrations for selected sites in the USA and Japan are shown in Fig. 1 and 2, respectively. These variations are caused by meteorological factors and to a lesser degree by seasonal changes in emission rates. Ambient temperature, wind speed, and inversion height are important factors affecting the dilution of air

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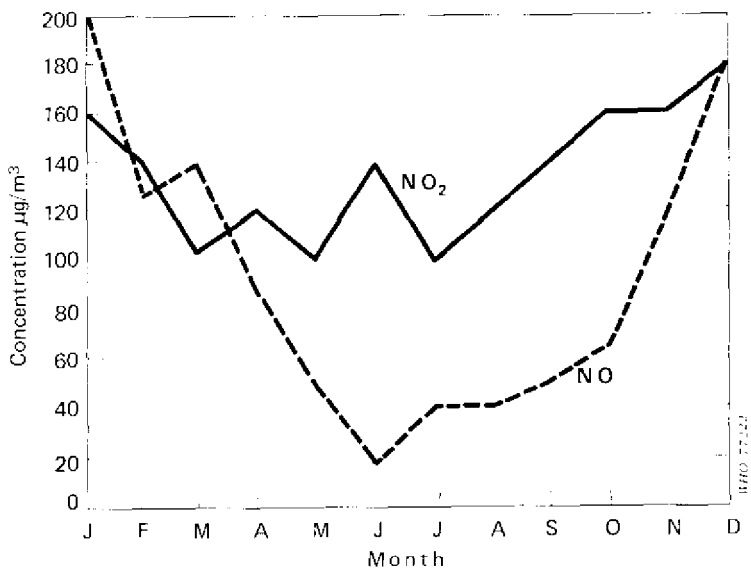
Fig. 1.   Monthly means of daily maximum nitric oxide and nitrogen dioxide concentrations in Los
Angeles Basin for 1962
(From: Schuck et al., 1966).
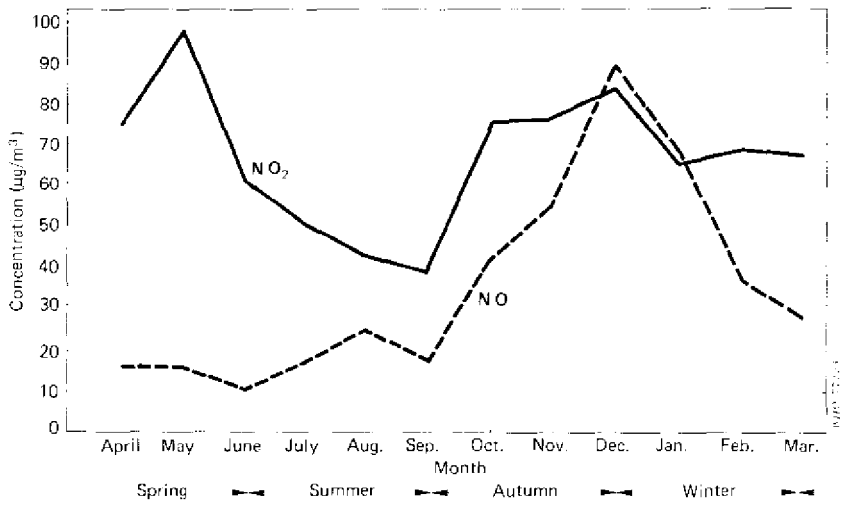
Fig. 2.   Monthly mean nitric oxide and nitrogen dioxide concentrations in Japan
(Shinjuku, Tokyo, April 1973–March 1974)
(From: Environment Agency, 1974).

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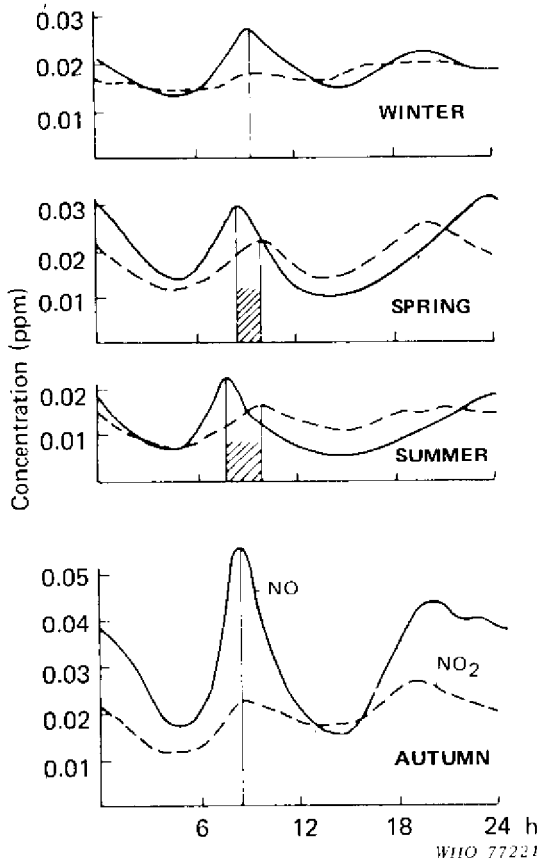
Fig. 3. Diurnal trends in nitric oxide and nitrogen dioxide concentrations in Delft (From: Guicherit, 1975).

pollutants. In addition, variations in nitrogen dioxide production by the atmospheric chemical reactions discussed in section 2 play a substantial role in the seasonal changes observed. In the cities cited, mean winter nitrogen dioxide values were 2 3 times higher than summer concentrations. Considering the complexity of the factors involved, this observation is probably not universal and other sites may show the opposite trends.

The frequency of occurrence of nitrogen dioxide hourly maximum concentrations in an urban area in the USA is shown in Table 7 (California Air Resources Board, 1975). During the period cited, the hourly maximum concentration of nitrogen dioxide exceeded 200 $\mu g/m^3$ (0.11 ppm) on more than 50% of the days. On one day a 1-h value of 839 $\mu g/m^3$ (0.46 ppm) was observed which is similar to the high 1-h value noted in Table 5 for Tokyo.

Table 7. Distribution of hourly maximum concentrations of nitrogen dioxide during July–September 1975 [a]

Site	Number of days with hourly maximum in nitrogen dioxide concentration range ($\mu g/m^3$)			
	200 400	400–600	600 800	>800
Los Angeles	43	5	2	1
Azusa	51	2	0	0
Burbank	52	9	1	0

[a]From: California Air Resources Board, 1975.

An example of urban, diurnal, seasonal variations in nitric oxide and nitrogen dioxide concentrations is given in Fig. 3 with reference to Delft in the Netherlands (Guicherit, 1975).

Features of interest include 2 peak concentrations of both nitric oxide and nitrogen dioxide found in the morning and evening which can be ascribed to the influence of automotive sources and occur typically on clear days. A time shift in the nitrogen dioxide peak is shown during spring and summer indicating increased photochemical conversion of nitric oxide into nitrogen dioxide.

Stationary sources involving fuel combustion for space heating can also produce early morning peaks.

4.3 Indoor Exposure

Exposure to oxides of nitrogen in the home due to the use of gas-fired appliances is usually underestimated. The recent expansion in the use of natural gas may have increased this exposure. Measurements conducted by Schwarzbach (1975) concerning nitrogen dioxide formation by gas-

fired domestic appliances such as space heaters, boilers, and cookers showed concentrations of up to 2000 $\mu g/m^3$ (1.1 ppm) at breathing height in the immediate vicinity of cookers.

The concentrations of nitrogen dioxide measured in a normally ventilated room using an oil-fired stove ranged from 380 to 1700 $\mu g/m^3$ (0.2–0.9 ppm) depending on the type of stove and from 750 to 940 $\mu g/m^3$ (0.4–0.5 ppm) when a gas-fired stove was used (Watanabe et al., 1966). Occupational exposure is discussed in section 6.2.

4.4 Smoking

Special mention must be made of the intense, deliberate exposure of man to oxides of nitrogen in tobacco smoke. Bokhoven & Niessen (1961) reported that tobacco smoke contained nitric oxide and nitrogen dioxide levels of 98–135 mg/m³ (80–110 ppm) and 150–226 mg/m³ (80–120 ppm)[b], respectively. This is equivalent to a nitric oxide intake of 160–500 µg per cigarette (Horton et al., 1974).

Haagen-Smit et al. (1959) made no distinction between nitric oxide and nitrogen dioxide in reporting oxides of nitrogen levels of 145–655 ppm in tobacco smoke.

5. EFFECTS ON EXPERIMENTAL ANIMALS

A considerable amount of toxicological data is available relating exposure to nitrogen dioxide with a variety of respiratory effects. The purpose of this section is to review and summarize selected animal studies which are most relevant for the evaluation of the health hazards resulting from exposure to nitrogen dioxide.

Very few studies have been reported on the effects of nitric oxide on experimental animals and, even in the most recent studies, the concentrations used have been much higher than ambient air levels (Greenbaum et al., 1967; Oda et al., 1975; Wagner, 1977, unpublished data[a]). Thus, the following discussion has been almost entirely limited to studies on the effects of exposure to nitrogen dioxide.

[a] Report on research work performed under the USA/Federal Republic of Germany Cooperative Program in Natural Resources, Environmental Pollution and Urban Development. Institut für Wasser-, Boden-, und Lufthygiene des Bundesgesundheitsamtes, 1977.

[b] According to G. Freeman, the values given for nitrogen dioxide are too high. He considers levels of 19–95 mg/m³ (10–50 ppm) to be more probable (Personal communication, 1977).

5.1 Local Effects on the Respiratory System

5.1.1 Morphological changes

There are several reports that describe alterations in the morphological integrity of the lung after exposure to nitrogen dioxide concentrations of 1900 μg/m^3 (1.0 ppm) and below. Šalamberidze (1969) did not find any pathological or histological changes in rats exposed for 90 days to a nitrogen dioxide level of 100μg/m^3 (0.05 ppm). However, electron microscopic studies by Buell (1970) revealed damage to insoluble collagen fibres isolated from the lungs of rabbits exposed to a nitrogen dioxide level of 470 μg/m^3 (0.25 ppm) for 4 h/day, 5 days/week, for 24–36 days.

Jakimčuk & Čelikanov (1968) reported that continuous exposure of rats to a nitrogen dioxide concentration of 600 μg/m^3 (0.32 ppm) for 90 days resulted in morphological changes such as peribronchitis, bronchitis, and light pneumosclerosis. Similar studies with a nitrogen dioxide concentration of 150 μg/m^3 (0.08 ppm) did not produce significant changes.

Inhalation of nitrogen dioxide concentrations of 1900 μg/m^3 (1.0 ppm) for 1 h or 940 μg/m^3 (0.5 ppm) for 4 h led to significant morphological changes in the mast cells of the lung in rats (Thomas et al., 1967). In exposed animals, the cells were ruptured and there was evidence of loss of cytoplasmic granules. These changes, which were observed in the pleura, bronchi, and surrounding tissue with more marked effects around the mediastinum, were reversible in 24 h.

Sherwin & Carlson (1973) found a relative increase in protein content in the lung lavage fluid of guineapigs continuously exposed to a nitrogen dioxide level of 750 μg/m^3 (0.4 ppm) for 1 week in comparison with that of control animals. While the meaning of the elevated protein levels is not yet clear, the authors believe that both protein leakage from the capillary bed and the increased rate of cell turnover within the exposed lung were responsible.

Blair et al. (1969) exposed mice to a nitrogen dioxide concentration of 940 μg/m^3 (0.5 ppm) for 6, 18 and 24 h daily and studied the sequential alterations in lung morphology. After 3–12 months, the alveoli were expanded in all exposed mice. The authors stated that the overall lesions appeared to be consistent with the development of early focal emphysema. Inflammation of the bronchioles, surface erosion of the epithelium, and blockage of the bronchiolar-alveolar junction were also observed.

Continuous exposure of mice to nitrogen dioxide concentrations of 940–1500 µg/m³ (0.5–0.8 ppm) for 1 month produced numerous structural changes. These effects included proliferation of the epithelial cells in the mucous membrane; degeneration and ablation of mucous membranes; oedematous changes in alveolar epithelial cells; shortening of cilia; and influx of monocytes (Hattori et al., 1972; Nakajima et al., 1969). Chen et al. (1972) studied recovery processes after nitrogen dioxide exposure. Immediately following exposure to nitrogen dioxide levels of 1900–2800 µg/m³ (1.0–1.5 ppm) for 1 month, the histological changes in exposed mice were identical to those reported above. However, when the animals were allowed to recover in clean air for 1–3 months there was a pronounced infiltration of lymphocytes around the brochioles which was not found in mice killed either during or immediately following exposure to nitrogen dioxide. The authors suggested that this response resembled those of an autoimmune disease.

Freeman et al., (1966) found slight bronchiolar, epithelial hypertrophy and the development of a moderate degree of tachypnea in rats continuously exposed for 33 months (approximately natural life-time) to a nitrogen dioxide level of 1500 µg/m³ (0.8 ppm). The authors repeated these long-term exposure studies at a concentration of 3800 µg/m³ (2.0 ppm). (Freeman et al., 1968a, 1968b, 1969; Freeman, 1970; Stephens et al., 1971a, 1971b, 1972). Exposure of rats to this concentration of nitrogen dioxide resulted in a number of microscopic and ultrastructural changes in the terminal bronchioles, alveolar ducts, and alveoli. The lungs were about 10% heavier than normal and the animals continued to exhibit tachypnea. There was homogeneous and uniform hypertrophy of the bronchiolar epithelium, loss of bronchiolar cilia, depression of natural cellular exfoliation, and blebbing of bronchiolar cells. Intracytoplasmic, crystalloid inclusion bodies appeared later. Electron microscopy revealed thickening of lung collagen fibrils and of the alveolar basement membranes.

Cell renewal rates were also studied in rats exposed to nitrogen dioxide (Evans et al., 1972, 1973a, 1973b, 1975), by measuring the uptake of tritiated thymidine by actively dividing alveolar cells. Continuous exposure to 3800 µg/m³ (2.0 ppm) caused a marked increase in number of type 2 alveolar cells. The labelling index reached a maximum at 48 h and by the seventh day had returned to its normal baseline level.

Monkeys (*Macaca speciosa*) exposed continuously for 14 months to a nitrogen dioxide concentration of 3800 µg/m³ (2.0 ppm) developed hypertrophy of the bronchiolar epithelium. Mixing an aerosol of sodium chloride at 330 µg/m³ with the nitrogen dioxide did not appear to alter

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the response (Furiosi et al., 1973). Several species of laboratory animals were also exposed to nitrogen dioxide levels of 19 mg/m^3 (10.0 ppm) or more in order to evaluate effects which could possibly lead to chronic obstructive pulmonary disease. At nitrogen dioxide levels of 19–47 mg/m^3 (10–25 ppm) for 26 and 13 weeks respectively, rats developed large, air-filled lungs that did not collapse under atmospheric pressure. The lungs became grossly emphysematous and the thoracic cage enlarged with dorsal kyphosis (Freeman & Haydon 1964; Freeman et al., 1968a, 1968b, 1969). Connective tissue changes involving both collagen and elastic tissue were observed. Animals began to die of respiratory failure after 16 months.

For further information and for detailed descriptions of morphological effects at these high concentrations the following publications are suggested: Freeman & Haydon (1964); Kleinerman & Cowdrey (1968); Kleinerman & Wright (1961); Parkinson & Stephens (1973).

5.1.2 Functional changes

Both short-term and long-term exposure to concentrations of nitrogen dioxide exceeding 1500 μg/m^3 (0.8 ppm) have been reported to cause changes in pulmonary function.

Rats exposed for 990 days to a nitrogen dioxide concentration of 1500 μg/m^3 (0.8 ppm) maintained elevated respiratory rates throughout their life (Freeman et al., 1966; Haydon et al., 1965).

Beagles exposed daily to a mixture of nitrogen dioxide and nitric oxide at approximate levels of 1210 μg/m^3 (0.64 ppm) and 310 μg/m^3 (0.25 ppm) respectively, for 61 months demonstrated reductions in both diffusion capacity and peak expiratory flow rates (Lewis et al., 1974). However, exposure to mixtures of nitrogen dioxide at 940–1900 μg/m^3 (0.5–1.0 ppm) and nitric oxide at 250 μg/m^3 (0.2 ppm) for 16 h per day, for 72 weeks, did not result in any changes in carbon monoxide diffusion capacity, compliance, or total expiratory resistance to airflow (Vaughan et al., 1969).

Neither transpulmonary resistance nor compliance was affected in rats exposed to 3800 μg/m^3 (2.0 ppm) throughout their lifetimes (approx. 2 years) although tachypnoea was consistently present (Freeman et al., 1968c). Nonhuman primates also breathed more rapidly when exposed for over 7 years to nitrogen dioxide levels of 3800 μg/m^3 (2.0 ppm) (Freeman & Juhos, 1976, Freeman et al., 1969) or to 9400 μg/m^3 (5.0 ppm) for 2 months (Henry et al., 1970). In the latter study, tidal volumes were also significantly reduced.

Guineapigs exposed to 9400 μg/m^3 (5 ppm) for $7\frac{1}{2}$ h per day, 5 days per week for $5\frac{1}{2}$ months showed no changes in expiratory flow resistance

(Balchum et al., 1965). Murphy et al., (1964) exposed guineapigs to 9800 $\mu g/m^3$ (5.2 ppm) for 4 h and recorded increased respiratory rate and decreased tidal volumes. Pulmonary function returned to normal when the animals were returned to clean air.

Rats exposed to a nitrogen dioxide level of 5500 $\mu g/m^3$ (2.9 ppm) for 24 h each day, 5 days per week for 9 months showed a significant decrease (13%) in lung compliance compared with controls (Arner & Rhoades, 1973). However, Wagner and co-workers (1965) were unable to detect any significant effects in rabbits exposed to a nitrogen dioxide concentration of 9400 $\mu g/m^3$ (5 ppm) for 6 h daily over a period of 18 months.

Davidson et al. (1967) exposed rabbits for 24 h/day for 3 months to nitrogen dioxide levels of 15–22.6 mg/m^3 (8–12 ppm) and observed reversible increases in non-elastic resistance and in functional residual capacity as well as a diminution in compliance.

The dose-effect relationship was studied in the lungs of rats and cats exposed to nitrogen dioxide concentrations of 940–38 000 $\mu g/m^3$ (0.5–20 ppm) (Zorn, 1975). A tendency towards an increase in respiratory rates and a decrease in arterial oxygen pressure was shown at concentrations as low as 1900 $\mu g/m^3$ (1.0 ppm). A single 2-h exposure to nitrogen dioxide levels of 19, 28, 66, and 94 mg/m^3 (10, 15, 35, and 50 ppm) affected the pulmonary function in squirrel monkeys. At concentrations of 19–28 mg/m^3 (10–15 ppm) the tidal volume decreased with little change in respiratory rate (Henry et al., 1969).

5.1.3 Biochemical effects

Nakajima & Kusumoto (1968) reported an initial reduction in the quantity of reduced glutathione in the lung and liver of mice exposed to a nitrogen dioxide concentration of 1500 $\mu g/m^3$ (0.8 ppm) continuously for 5 days. On the fifth day, the level of glutathione approached normal and was no longer significantly different from that of the controls. However, with continuous exposure over 6 months the animals tended to lose weight and the glutathione level fell once more (Nakajima et al., 1969, Nakajima, 1973).

Chow et al. (1974) observed a rise in glutathione peroxidase (1.11.1.9) activity in the lungs of rats exposed to nitrogen dioxide concentrations of 1900 and 4300 $\mu g/m^3$ (1.0 and 2.3 ppm) for 4 days. At about 12 mg/m^3 (6.2 ppm) there was a significant increase in the activities of glutathione reductase (NAD(P)H) (1.6.4.2) and glucose-6-phosphate dehydrogenase

(1.1.1.49) in comparison with the controls. The authors believe that alterations in such enzyme systems are sensitive and specific bioindicators of tissue damage.

Fukase et al. (1976) reported that exposure to nitrogen dioxide at about 11 mg/m³ (6 ppm) for 4 h every day for 30 days caused an increase in glutathione reductase (NAD(P)H) (1.6.4.2) and glucose-6-phosphate dehydrogenase (1.1.1.49) activities. Exposure to a nitrogen dioxide level of 28 mg/m³ (15 ppm) for 7 days resulted in a significant increase in glutathione levels. Exposure to 53 mg/m³ (28 ppm) for 7 days resulted in significant increases in glutathione levels and in the activities of glutathione reductase (NAD(P)H) (1.6.4.2), glucose-6-phosphate dehydrogenase (1.1.1.49) and glutathione peroxidase (1.11.1.9).

Biochemical evidence indicating that on exposure to nitrogen dioxide there is a proliferation of type 2 alveolar cells to replace the injured type 1 cells in lung tissue has been submitted by Sherwin et al. (1972). In these investigations, guineapigs were exposed to a concentration of 3800 µg/m³ (2 ppm) continuously for 1–3 weeks; a significant increase occurred in the lactate dehydrogenase (cytochrome) (1.1.2.3) index of the lower lobes.

Oxygen consumption and lactate dehydrogenase (cytochrome) (1.1.2.3), and aldolase (4.1.2.13) activity levels were all elevated in lung, liver, kidney, and spleen tissue following short-term and long-term exposure of guineapigs to nitrogen dioxide (Buckley & Balchum 1965, 1967a, 1967b). In the short-term treatment the guineapigs exposed to 75 mg/m³ (40 ppm) for a total of 4½ h were killed 2 h after treatment. The long-term treatment included exposure to 28 mg/m³ (15 ppm) continuously for 10 weeks. The mechanisms involved in these changes have not yet been indentified but they may reflect an acute response to stress.

By applying the disc electrophoresis method, Sherwin & Carlson (1973) demonstrated higher protein levels in the lavage fluid of guineapigs exposed for 1 week to a nitrogen dioxide concentration of 750 µg/m³ (0.4 ppm).

Thomas et al. (1968) found that short-term exposure (4 h) to 1900 µg/m³ (1.0 ppm) resulted in the lipoperoxidation of lung lipids in rats. Rats fed on a vitamin E-deficient diet and then exposed to nitrogen dioxide had more peroxidation of surfactant and tissue lipids than did rats on a vitamin E-supplemented diet (Roehm et al., 1971). Antioxidants appeared to serve as protection against peroxidation and free radical formation (Menzel et al., 1972).

Arner & Rhoades (1973) reported a significant decrease (8.7%) in the lung lipid content of rats exposed to a nitrogen dioxide concentration of 5500 µg/m³ (2.9 ppm) for 9 months and also a marked decrease in the

percentage of total saturated phospholipid fatty acids. This reduction in saturation was primarily due to a decrease in the percentage of hexadecanoic (palmitic) acid. There were also significant changes in the surface properties of the lung washings from animals exposed to nitrogen dioxide indicating an increase in surface tension and a decrease in stability of the pulmonary surfactant. Continuous exposure for 14 days to a nitrogen dioxide concentration of 9400 µg/m³ (5.0 ppm) markedly decreased the lecithin turnover rate in rat lungs (Thomas & Rhoades, 1970). The authors suggested that the pulmonary phospholipid synthesis might be altered by nitrogen dioxide exposure.

5.2 Other Effects

Although the primary target for nitrogen dioxide exposure is the lung, data are accumulating that indicate that it may effectively alter a wide range of other systems.

5.2.1 Effects on growth and body weight

Numerous investigators have measured the growth rate of animals during exposure to nitrogen dioxide and have produced conflicting results. Body weights of rats and hamsters were reported to be significantly lower than those of the controls in studies by: Kaut et al. (1966) with a nitrogen dioxide exposure of 5000 µg/m³ (2.7 ppm) for 8 weeks; Freeman & Haydon (1964) with a concentration of 24 mg/m³ (12.5 ppm) for 213 days; and Kleinerman & Rynbrandt (1976) with a concentration of 38 mg/m³ (20 ppm) for 24 h. Other investigators using guineapigs, hamsters, mice, rabbits, dogs, and rats failed to find any such effects (Nakajima et al. (1969) with nitrogen dioxide exposures of 1300 1500 µg/m³ (0.7 0.8 ppm) for 1 month; Šalamberidze (1969) with a concentration of 100 µg/m³ (0.05 ppm) for 90 days; and Wagner et al. (1965) with a concentration of 1900 47 000 µg/m³ (1.0–25 ppm) for 18 months).

5.2.2 Immunological effects

Nitrogen dioxide exposure seems to alter the immunological reaction of experimental animals. Continuous exposure to 9400 µg/m³ (5.0 ppm) for 3 -5 months appeared to depress the squirrel monkey's ability to form protective serum neutralizing antibodies. However, when monkeys were exposed for 16 months to a nitrogen dioxide concentration of 1900 µg/m³

(1.0 ppm), the exposed animals consistently showed higher serum neutralizing antibody titres than the control (Ehrlich & Fenters, 1973; Fenters et al., 1971). The same workers (Ehrlich et al., 1975) exposed mice to 940 µg/m³ (0.5 ppm) continuously while superimposing a 1-h peak of 3800 µg/m³ (2.0 ppm) each day over a period of 3 months in order to determine if this exposure produced changes in the circulating immunoglobulins. Non-vaccinated mice showed a marked decrease in levels of IgA and an increase in serum IgM, IgG1, and IgG2. These investigators also measured haemagglutination-inhibition (HI) and serum neutralization (SN) antibody formation and found that nitrogen dioxide depressed the SN antibody formation but did not alter the HI titres.

Contrary to these findings, Antweiler et al. (1975) did not find any alteration in the ability of the guineapig to produce antibodies, even after 33 days of exposure to 10 mg/m³ (5.3 ppm).

A month of continuous exposure to a nitrogen dioxide level of 1700 µg/m³ (0.9 ppm) reduced the ability of the mouse spleen to produce primary antibodies (Nakamura et al., 1971).

Balchum et al. (1965) reported a circulating substance in the serum of guineapigs exposed to nitrogen dioxide which had properties similar to a lung antibody. This substance reacted *in vitro* with proteins extracted from the lung tissue of control animals. The titres of this reactive substance increased with the intensity and duration of exposure to nitrogen dioxide. Exposure concentrations were 9400 and 28 000 µg/m³ (5 and 15 ppm) for periods of up to 1 year.

5.2.3 Haematological effects

Šalamberidze (1969) studied the effects of continuous exposure (24 h/day) of rats to a nitrogen dioxide concentration of 100 µg/m³ (0.05 ppm) for periods of up to 90 days. The author did not find any effects on haemoglobin or erythrocytes.

Polycythemia, with reduced mean corpuscular volume but a normal mean corpuscular haemoglobin concentration was found in rats and monkeys (*Macaca speciosa*) following continuous exposure for 3 months to 3800 µg/m³ (2.0 ppm) (Furiosi et al., 1973). Mitina (1962) reported leucocytosis in rabbits exposed to nitrogen dioxide concentrations of 2400–5700 µg/m³ (1.3–3.0 ppm) for 2 h per day for 15 and 17 weeks. This effect was followed by a phagocytic depression of circulating leucocytes. The leucocytic response was accelerated by the presence of sulfur dioxide.

Carson et al. (1962) exposed dogs to nitrogen dioxide concentrations of 73–310 mg/m³ (39–164 ppm) for periods ranging from 5 min to 1 h.

They did not find any changes in haematocrit or blood platelet counts 4, 24, 48, or 72 h after exposure. Wagner et al. (1965) did not find any haematological effect when dogs were exposed for 18 months to nitrogen dioxide concentrations of 1900 or 9400 $\mu g/m^3$ (1 or 5 ppm).

The addition of nitrogen dioxide at concentrations of 940–1500 $\mu g/m^3$ (0.5–0.8 ppm) to carbon monoxide at 58 mg/m^3 (50 ppm) failed to affect the carboxyhaemoglobin concentrations in the blood of mice in studies reported by Nakajima & Kusumoto (1970).

The chemical action of nitrogen dioxide on the circulating erythrocyte *in vivo* is poorly understood. Although methaemoglobinaemia could possibly result from exposure to low levels of nitrogen dioxide, evidence confirming this is not available. Wagner (1977, unpublished data [a]) could not find a detectable increase in the concentration of methaemoglobin in rats exposed to 9400 $\mu g/m^3$ (5 ppm) for as long as 10 months. However, exposure to 19 mg/m^3 (10 ppm) for 1 h induced a significant increase in the concentration of methaemoglobin in arterial blood. Nakajima & Kusumoto (1968) did not find any increase in the concentration of methaemoglobin in the blood of mice continuously exposed to a nitrogen dioxide concentration of 1500 $\mu g/m^3$ (0.8 ppm) for 5 days.

5.2.4 Miscellaneous biochemical effects

Continuous exposure of rats to a nitrogen dioxide concentration of 100 $\mu g/m^3$ (0.05 ppm) for 90 days did not produce any effects on the activities of cholinesterase (3.1.1.8), catalase (1.11.1.6), and SH-groups in blood (Šalamberidze, 1969).

Veninga & Lemstra (1975) reported that a single 2-h exposure to 560–7500 $\mu g/m^3$ (0.3–4.0 ppm) produced elevated levels of ascorbic acid in the liver of mice.

Kosmider & Misiewicz (1973) exposed guineapigs to a nitrogen dioxide concentration of 1900 $\mu g/m^3$ (1.0 ppm), continuously, for a total of 180 days. The authors observed increased aminotransferase activity in the blood serum and heart homogenates but decreased levels in the brain and in the liver. No significant alterations were evident in the basic alkaline phosphatase (3.1.3.1) and magnesium-activated phosphatase activities in the blood serum of dogs exposed to nitrogen dioxide levels of either 1900 or 9400 $\mu g/m^3$ (1 or 5 ppm) for 18 months (Wagner et al., 1965).

However, Wagner (1972) found an elevation in serum cholesterol in rats after continuous exposure to 9400 $\mu g/m^3$ (5 ppm) for 1 year.

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[a]See footnote, p. 32.

Drozdz and co-workers (1973, 1974, 1975) measured the effects of 6 months continuous exposure to a nitrogen dioxide concentration of 2000 $\mu g/m^3$ (1.1 ppm) on the guineapig. They studied alterations in the activity of various enzymes in both the blood and liver as well as in the central nervous system. The experiments demonstrated that prolonged exposure to nitrogen dioxide led to disturbances in the levels of glucose, lactic acid, total lipids, seromucoids, hexoses, hexamines, and sialic acid. Kaut et al. (1966) reported a decrease in the albumin/globulin (A/G) quotient as well as in the vitamin C content of the suprarenal glands, in rats exposed to a nitrogen dioxide concentration of 5000 $\mu g/m^3$ (2.7 ppm) for 2 8 weeks, 6 h per day, 5 days per week.

Continuous exposure to high nitrogen dioxide concentrations of 28 216 mg/m^3 (15 115 ppm) produced numerous systemic biochemical changes. Nitrogen dioxide has been reported to produce increases in: serum protease inhibitor activity; plasma corticosterone levels; oxygen consumption in the spleen and kidney; lactate dehydrogenase (cytochrome) (1.1.2.3) activity in liver and kidney; and aldolase (4.1.2.13) activity in the liver, kidney, spleen, and serum (Buckley & Balchum, 1965; Kleinerman & Rynbrandt, 1976; Tusl, 1975). Švorcová & Kaut (1971) reported an elevation in urinary nitrites and nitrates in rabbits immediately after exposure for 15 min to a concentration of 45 mg/m^3 (23.9 ppm). It is possible that this indicates that nitrogen dioxide is rapidly converted to nitrite and nitrate ions and that these ions are excreted in the urine shortly afterwards.

5.2.5 Effects on reproduction

Šalamberidze & Cereteli (1971) studied changes in the female rat's reproductive and endocrine systems resulting from exposure to 2360 $\mu g/m^3$ (1.3 ppm), 12 h per day for 3 months. There was a prolongation of the estrus cycle associated with an increased interestrual period and a decrease in the number of monthly cycles. The litter size and the fetal weights decreased but the capacity for pregnancy was not affected. These effects may reflect the effect of nitrogen dioxide exposure on endocrine or reproductive function.

5.2.6 Effects on the central nervous system

Šalamberidze (1969) did not find any effects on the central nervous system in rats exposed to a nitrogen dioxide concentration of 100 $\mu g/m^3$ (0.05 ppm) for 90 days.

At a slightly higher concentration, Jakimčuk & Čelikanov (1968) reported a significant delay in the conditioned reflexes of the central nervous system in rats after 90 days of exposure to a nitrogen dioxide concentration of 600 μg/m³ (0.32 ppm).

5.2.7 Behavioural changes

Murphy et al. (1964) reported that the voluntary running activity of male mice was depressed when the concentration of nitrogen dioxide reached 14 mg/m³ (7.7 ppm) and the animals were exposed for 6 h. A similar loss in activity was reported by Tusl et al. (1973). They measured the influence of nitrogen dioxide on the performance of rats during physical exertion as measured by swimming. In rats exposed to 9400 μg/m³ (5.0 ppm), a decrease of 25% in performance occurred in the fifth to sixth week of the experiment. Animals exposed to 1900 μg/m³ (1.0 ppm) also showed a tendency towards decreased performance, although this was not statistically significant.

5.2.8 Carcinogenicity, mutagenicity, and teratogenicity

In order to study the possible formation of nitrosamines by the reaction of nitrogen dioxide with tissue amines, mice were exposed to a nitrogen dioxide concentration of 75 mg/m³ (40 ppm) for periods up to $1\frac{1}{2}$ years. Although proliferative alterations at the terminal bronchioles were always present, no carcinomas were found in the lungs of these animals (Henschler & Ross, 1966).

Kaut (1970) analysed lung tissue to detect nitro- and nitroso-compounds, especially nitrosamines in white rats exposed to mixtures of oxides of nitrogen ranging from 5 to 250 ppm for 3 h. The compounds were found *in vitro* in tissues exposed to high concentrations of oxides of nitrogen, but not *in vivo*.

Rats were exposed for periods ranging from $2\frac{1}{2}$ months to a lifetime (2 years) to automotive exhaust gas containing carbon monoxide, oxides of nitrogen, carbon dioxide, and aldehydes at concentrations of 58 mg/m³ (50 ppm), 23 ppm, 6700 mg/m³ (3700 ppm), and 2.0 ppm, respectively. According to the author, spontaneous tumours and abscesses were more frequent in the group exposed to the gas than in the control group but none occurred in the lung tissue (Stupfel et al. 1973).

These studies cannot be considered to provide any evidence of the carcinogenic effect of oxides of nitrogen.

No evidence is available on the mutagenicity and teratogenicity of oxides of nitrogen *per se*, but nitrous acid has been reported to be mutagenic in some laboratory tests.

5.3 Interaction of Nitrogen Dioxide and Infectious Agents

The influence of nitrogen dioxide on susceptibility to respiratory infection and its adverse effect on the pulmonary defence system of the host has been clearly demonstrated in several species of animals.

Ehrlich (1966) and Henry et al. (1969) showed that exposure of mice, hamsters, and squirrel monkeys to nitrogen dioxide made them more vulnerable to respiratory infection with *Klebsiella pneumoniae*, the mouse being the most sensitive. With a 2-h exposure, the minimum concentration of nitrogen dioxide required to produce a significant rise in the mortality rate was 6600 $\mu g/m^3$ (3.5 ppm). No effect was observed at 4700 $\mu g/m^3$ (2.5 ppm). However, when mice were exposed continuously for 1 year to 940 $\mu g/m^3$ (0.5 ppm), a statistically significant increase in mortality rate occurred after 90 days (Blair et al., 1969; Ehrlich & Henry, 1968).

Ito et al. (1971) exposed female mice to nitrogen dioxide concentrations of 940 1900 $\mu g/m^3$ (0.5 1.0 ppm) continuously for 39 days and studied the influence of the nitrogen dioxide on infection with influenza virus histopathologically. Advanced interstitial pneumonia and adenomatous proliferation in the epithelium of the peripheral bronchi were noted. Intermittent exposure to 19 mg/m^3 (10.0 ppm) for 2 h daily, for 5 days, also significantly increased the susceptibility of mice to influenza virus infection as demonstrated by increased mortality.

Motomiya et al. (1972) studied the interaction of nitrogen dioxide and the influenza virus. They reported a high incidence of adenomatous proliferation of peripheral, bronchial, epithelial cells in mice exposed for 3 months to nitrogen dioxide levels of 560 940 $\mu g/m^3$ (0.3 0.5 ppm) followed by infection with the influenza virus. The effect was more serious than that seen in infected mice kept in clean air. Continuous exposure for an additional 3 months did not enhance the effect.

Continuous exposure of squirrel monkeys to nitrogen dioxide levels of 9400 $\mu g/m^3$ and 19 mg/m^3 (5 and 10 ppm) for 1 or 2 months increased their susceptibility to both bacterial and viral infections. All the animals exposed to 19 mg/m^3 (10 ppm) died within 2–3 days of infection with the influenza virus. At 9400 $\mu g/m^3$ (5 ppm) 1 of the 3 experimental monkeys died. All control monkeys had symptoms of viral infection but no deaths occurred. When the nitrogen dioxide exposed monkeys were challenged with *Klebsiella pneumoniae*, 2 out of 7 monkeys exposed to 9400 $\mu g/m^3$ (5 ppm) for 2 months died and the remainder had the infectious agent in the lungs at autopsy. At 19 mg/m^3 (10 ppm) for 1 month 1 of 4 monkeys died and 2 had the infectious agent in the lungs at autopsy (Henry et al.,

1969, 1970). Both squirrel monkeys and hamsters showed a reduction in resistance to *Klebsiella pneumoniae* after a single 2-h exposure to nitrogen dioxide concentrations of 66–75 mg/m^3 (35–40 ppm) (Ehrlich 1966).

Gardner et al. (1977) and Coffin et al. (1976) studied the time-dose-response for nitrogen dioxide— *Streptococcus pyogenes* interaction. Mice were exposed to nitrogen dioxide concentrations ranging from 940 µg/m^3–53 mg/m^3 (0.5–28 ppm) for various periods of time ranging from 10 min to 12 months before treatment with the bacterial aerosol. When comparisons were made, it was evident that the mortality rate increased with increasing concentrations of nitrogen dioxide. Different relationships between concentration and time produced significantly different mortality responses. The data suggest that concentration has a greater influence on the effect of nitrogen dioxide than length of exposure.

Studies were also conducted to compare continuous with intermittent exposure to nitrogen dioxide at concentrations of 2800 and 6600 µg/m^3 (1.5 and 3.5 ppm). There was a significant increase in mortality rate for each of the experimental groups with increasing duration of exposure. When the data were adjusted for the total difference in concentration x time, the mortality rate was essentially the same for both groups.

There are numerous studies that show that nitrogen dioxide is injurious to specific pulmonary defence systems in the lung. It interferes with the efficiency of clearing inhaled particles including bacteria and viruses from the airway and with the phagocytosis and digestion of such particles by the alveolar macrophage. As this is the major defence against infection by inhalation, any alteration in this system would increase the risk of infection (Green & Kass, 1964; Kass et al. 1966).

Aranyi & Ehrlich (1973, unpublished data [a]) isolated alveolar macrophages from mice continuously exposed to a nitrogen dioxide concentration of 4700 µg/m^3 (2.5 ppm) for 3 h daily, 5 days per week, for one month. Scanning electron microscopy revealed changes in the surface of these cells and a reduction in the ability of the cells to phagocytize *Escherichia coli, in vitro.*

Goldstein et al. (1973) measured the effect of nitrogen dioxide on antibacterial activity in the mouse. Pulmonary bactericidal activity decreased progressively with exposure to increasing concentrations of nitrogen dioxide. This defect was present in animals exposed for 4 h to concentrations of nitrogen dioxide of 13 mg/m^3 (7 ppm) or more. With exposure for 17 h, this bactericidal dysfunction occurred at levels as low as 4300 µg/m^3 (2.3 ppm). These reports are consistent with the earlier report of Gardner et al. (1969) who obtained macrophages from the

lungs of rabbits after *in vivo* phagocytosis and found a pronounced inhibition of phagocytic activity (50%) after a 3-h exposure to nitrogen dioxide at 19 mg/m^3 (10 ppm). A 2-h exposure to 15 mg/m^3 (8.0 ppm) increased the proportion of polymorphonuclear leucocytes in the lavage fluid. This condition persisted for more than 72 h after the cessation of exposure.

Acton & Myrvik (1972) found that rabbits exposed to nitrogen dioxide concentrations of 28 mg/m^3 (15.0 ppm) for a short period of time (3 h) had alveolar cells with a lower capacity to develop virus-induced resistance and to phagocytize BCG vaccine. Valand et al. (1970) demonstrated that macrophages washed from the lungs of rabbits exposed for 3 h to a nitrogen dioxide concentration of 47 mg/m^3 (25 ppm) and injected with parainfluenza-3 virus failed to develop resistance to rabbit pox virus. The alveolar macrophages obtained from these exposed animals failed to produce interferon.

Buckley & Loosli (1969) exposed mice for 6 weeks to a nitrogen dioxide concentration of 71 mg/m^3 (38.0 ppm) and were unable to detect any alteration in the rate of clearance of an aerosol of staphylococci.

The second mechanism of host defence is the mechanical or physical removal of inhaled and deposited particles by means of the mucociliary escalator.

Giordano & Morrow (1972) determined that exposure to a nitrogen dioxide concentration of 11 mg/m^3 (6 ppm) continuously for 6 weeks depressed this mucociliary transport.

5.4   Summary Table

Experimental animal studies which provide useful quantitative information for the establishment of guidelines for the protection of public health with respect to nitrogen dioxide exposure are summarized in Table 8.

6. EFFECTS ON MAN

6.1 Controlled Exposures

The effects of nitrogen dioxide on both healthy subjects and patients have been studied (with their consent) under controlled conditions. Although the studies are few in number and only concern short-term exposure, much useful information has been obtained for assessing health effects in man.

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Table 8. Experimental animal studies

1. Local effects on the respiratory system

Nitrogen dioxide concentration (µg/m³)	(ppm)	Length of exposure (number of days)	(h/day)	Effects	Response*	Species	Number of animals	Reference
1900	1.0	4	24	glutathione peroxidase (1,1,1,1,9) activity significantly increased in lung		rat	5 (11)	Chow et al. (1974)
1900	1.0	1	4	peroxidation of lung lipids		rat	6 (10)	Thomas et al. (1968)
1500	0.8	990	24	elevated respiratory rates throughout their lives	9/9 (0/12)	rat	9 (12)	Freeman et al. (1966)
1500	0.8	990	24	minimal bronchiolar epithelial hypertrophy	9/9 (0/12)	rat	9 (12)	Freeman et al. (1966)
1500	0.8	5	24	significant decrease in lung reduced glutathione		mouse	10 (10)	Nakajima & Kusumoto (1968)
940–1500	0.5–0.8	30	24	degeneration and desquamation of mucous membrane; shortening and reduction of the cilia of ciliated epithelial cells;	10/10 (0/5)	mouse	10 (5)	Nakajima et al. (1969)
940–1500	0.5–0.8	30	24	oedematous change of alveolar epithelial cells proliferation of epithelial cells of the peripheral bronchus (adenomatous changes)	6/10 (0/5)	mouse	10 (5)	Hattori et al. (1972)
1200 plus 310 nitric oxide	0.64 plus 0.25 nitric oxide	61 months	16	reduction in pulmonary diffusion capacity	6/11 (3/8)	dog	11 (18)	Lewis et al. (1974)
1200 plus 310 nitric oxide	0.64 plus 0.25 nitric oxide	61 months	16	decreased peak expiratory flow rate	5/11 (1/8)	dog	11 (18)	Lewis et al. (1974)
940 plus 250 nitric oxide	0.5 plus 0.2 nitric oxide	18 months	16	no changes in carbon monoxide diffusion capacity, compliance, or total expiratory resistance	0/12 (0/20)	dog	12 (20)	Vaughan et al. (1969)

Table (continued)

Concentration (µg/m³)	Concentration (ppm)	Duration (days)	Exposure (h/day)	Effect	Animals affected/total (controls)	Species	No. of animals	Reference
940	0.5	90	6, 18, 24	evidence of focal emphysema	12/12 (0/4)	mouse	12 (4)	Blair et al. (1969)
940	0.5	360	1 / 4	reduction in mitochondria of alveolar cells and degradation of mast cells	6/6 (0/6)	rat	6 (6)	Thomas et al. (1967)
750	0.4	7	24	significant increase in protein content of lung lavage fluid		guinea pig	9 (9)	Sherwin & Carlson (1973)
600	0.32	90	24	morphological changes such as bronchitis, peribronchitis, and light pneumosclerosis; no effect observed at 150 µg/m³ (0.08 ppm)		rat	15 (15)	Jakimčuk & Čelikanov (1969)
470	0.25	24–36	4	structural changes in lung collagen fibres (electron microscope)	2/3 (0/1)	rabbit	3 (1)	Buell (1970)
100	0.05	90	24	no pathological or histological effects	0/10 (0/10)	rat	10 (10)	Salamberidze (1969)

ii. Other effects

Concentration (µg/m³)	Concentration (ppm)	Duration	Exposure (h/day)	Effect	Species	No. of animals	Reference
1900	1.0	16 months	24	higher serum neutralizing antibody titres than control	squirrel monkey	5	Ehrlich & Fenters (1973)
1900	1.0	180	24	decreased aminotransferase activity in brain and liver, increased activity in blood serum and heart	guinea pig	30 (50)	Kosmider & Misiewicz (1973)
1700	0.9	30	24	reduction in antibody production in spleen	mouse	9 (9)	Nakamura et al. (1971)
1300–1500	0.7–0.8	1 month	24	no change in growth rate	mouse	20 (20)	Nakajima et al. (1969)
940 with 1 h peak (3800) daily	0.5 with 1 h peak (2) daily	3 months	24	changes in circulating immunoglobulins; depression in serum neutralizing antibody titres	mouse	112–160 (112–160)	Ehrlich et al. (1975)
940–1500 + 58 000 carbon monoxide	0.5–0.8 + 50 carbon monoxide	1–1.5 months	24	no change in blood carboxyhaemoglobin	mouse	94 (49)	Nakajima & Kusumoto (1970)
600	0.32	90	24	significant changes in conditioned reflexes of the central nervous system	rat	15 (15)	Jakimčuk & Čelikanov (1968)
560–7500	0.3–4.0	1	2	increase in ascorbic acid levels in liver	mouse	20 (114)	Veninga & Lemstra (1975)
100	0.05	90	24	no effects on weight gain, central nervous system, activities of cholinesterase (3.1.1.8), catalase (1.1.1.6) and SH-groups in blood, haemoglobin, or erythrocytes	rat	10 (10)	Salamberidze (1969)

Number of animals showing effects/total number of animals; numbers in brackets refer to control groups

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Table 8. Experimental animal studies – *continued*

III. Interaction with infectious agents

Nitrogen dioxide concentration		Length of exposure		Effects	Response [a]	Species	Number of animals	Reference
($\mu g/m^3$)	(ppm)	(number of days)	(h/day)					
940-1900	0.5-1.0	39	24	higher incidence of adenomatous proliferation of bronchial and bronchiolar epithelium than unexposed challenged group	7/12 (1/12)	mouse	12 (12)	Ito et al. (1971)
940	0.5	12 months	24	increased susceptibility to infection; first statistical significance evident at 90 days; reduced pulmonary clearance of inhaled microbes		mouse	4 (4)	Blair et al. (1969) Ehrlich & Henry (1968)
560-940	0.3-0.5	3 months	24	more severe adenomatous proliferation of the peripheral bronchial cells than unexposed challenged group		mouse	12 (8)	Motomiya et al. (1972)
560-940	0.3-0.5	6 months	24	no further enhancement by an additional 3 months exposure		mouse	12 (8)	Motomiya et al. (1972)

[a] Number of animals showing effects/total number of animals; numbres in brackets refer to control groups.

Henschler et al. (1960) studied normal, healthy males, aged 20–35 years to obtain data concerning the threshold of odour perception for nitrogen dioxide. When the concentrations reached 230 μg/m³ (0.12 ppm), 3 out of 9 subjects perceived the odour immediately and 8 out of 13 could detect concentrations of 410 μg/m³ (0.22 ppm). At a higher concentration of 790 μg/m³ (0.42 ppm), 8 out of 8 subjects immediately recognized the odour. When the nitrogen dioxide concentration was increased very gradually from 0 to 51 mg/m³ (27 ppm), the volunteers failed to detect the odour. Awareness of the odour increased when the humidity was increased from 60% to 80%. Similar studies on odour perception were carried out by Feldman (1974) and Šalamberidze (1967) and in these experiments the nitrogen dioxide olfactory thresholds were found to be 200 and 230 μg/m³ (0.11 and 0.12 ppm), respectively.

Threshold values for the impairment of dark adaptation by nitrogen dioxide have also been reported. Šalamberidze (1967) determined that the threshold after 5 and 25 min of nasal inhalation of nitrogen dioxide was 140 μg/m³ (0.074 ppm).

Studies with human volunteers were also made to determine subtle changes in respiratory function during nitrogen dioxide exposure. Volunteer patients with moderate degrees of chronic respiratory diseases were studied as well as healthy individuals.

Airway resistance increased significantly compared with pre-exposure resistance following 5-min exposure of 15 healthy individuals to nitrogen dioxide levels of 5600–75 000 μg/m³ (3–40 ppm) (Nakamura, 1964). Suzuki & Ishikawa (1965) exposed 10 healthy subjects to nitrogen dioxide concentrations ranging from 1300–3800 μg/m³ (0.7–2.0 ppm) for 10 min. The inspiratory and expiratory flow resistance rose to about 150 and 110% of control values, respectively, 10 min after the exposure.

Abe (1967) reported studies in which 5 healthy males were exposed to nitrogen dioxide levels of 7500–9400 μg/m³ (4–5 ppm) for 10 min. Inhalation of nitrogen dioxide caused an increase in both expiratory and inspiratory flow resistance reaching a maximum 30 min after the end of the exposure. Values for effective compliance obtained 30 min after cessation of exposure showed a 40% decrease compared with controls.

Orehek et al. (1976) measured the bronchomotor sensitivity of asthmatic patients to a bronchoconstrictor agent (carbachol) before and after exposure to nitrogen dioxide. These authors attempted to establish dose-response curves for the specific airway resistance (SR_{aw}) of 20 asthmatics who were exposed for 1 h to 190 and 380 μg/m³ (0.1 and 0.2 ppm). The degree of enhancement of bronchial sensitivity by the nitrogen dioxide was variable among the individuals tested. Nitrogen dioxide at 190 μg/m³ (0.1 ppm) induced a significant increase in initial

SR_{aw} and enhanced the bronchoconstrictor effect in 13 subjects. In 7 subjects this level of nitrogen dioxide did not modify either of these effects. Several possible reasons were advanced by the authors to explain why some asthmatic subjects responded and others did not. It seems clear that sensitivity to nitrogen dioxide can vary among individuals. Yokoyama (1968, 1970, 1972) also reported considerable individual variation in response among volunteers exposed from 10 to 120 min to several concentrations of nitrogen dioxide.

Nieding and his associates conducted a series of studies on the effects of nitrogen dioxide on pulmonary function in man.

In 1970, they reported pulmonary function studies on 13 healthy subjects and 88 patients with chronic bronchitis who were exposed for 15 min to nitrogen dioxide levels of 940–9400 $\mu g/m^3$ (0.5 5.0 ppm). Inhalation of concentrations below 2800 $\mu g/m^3$ (1.5 ppm) had no significant effect. Concentrations between 3000 and 9400 $\mu g/m^3$ (1.6 and 5.0 ppm) caused a significant increase in airway resistance in the patients with chronic bronchitis. The patients also reacted with a significant decrease in arterial oxygen pressure and an increase in the alveolar-arterial oxygen pressure gradient when they inhaled levels of 7500 9400 $\mu g/m^3$ (4 5 ppm). No effect was seen at 3800 $\mu g/m^3$ (2 ppm).

In these studies, exposure of healthy individuals to 9400 $\mu g/m^3$ (5.0 ppm) caused a significant decrease in arterial oxygen pressure while the end expiratory oxygen partial pressure remained unchanged. Increased end expiratory arterial oxygen pressure difference was accompanied by a significant increase in systolic pressure in the pulmonary artery (Nieding et al., 1970, unpublished data [a]).

Nieding et al., (1971) observed an elevation in airway resistance in 15 patients with chronic bronchitis following exposure to nitrogen dioxide concentrations of 3000-3800 $\mu g/m^3$ (1.6 2.0 ppm) for 15 min. At concentrations above 3800 $\mu g/m^3$ (2.0 ppm) the increase became more pronounced. Below a concentration of 2800 $\mu g/m^3$ (1.5 ppm), no significant changes were observed.

Inhalation of 9400 $\mu g/m^3$ (5.0 ppm) for 15 min caused a significant decrease in the carbon monoxide diffusing capacity of 16 healthy volunteers (Nieding et al., 1973a). When the alveolar partial pressures of oxygen before, during, and after inhalation of nitrogen dioxide were compared, the mean values for the 14 chronic bronchitis patients tested were not statistically different. However, the arterial oxygen pressure decreased from an average of 102 × 10^2 to 95 × 10^2 Pa during nitrogen

dioxide exposure. There was a corresponding significant increase in alveolo-arterial oxygen pressure gradients from an average of 34×10^2 to 43×10^2 Pa. Continued exposure for 60 min did not result in any further significant disturbances in respiratory gas exchange (Nieding et al., 1973a).

Nieding et al., (1977) exposed 11 healthy male subjects, aged 24–38 years, to a nitrogen dioxide level of 9400 $\mu g/m^3$ (5.0 ppm) for 2 h a day. Changes in pulmonary function were compared with t h pre- and post-control periods without nitrogen dioxide and with an untreated control series. Under test conditions, including intermittent light exercise, a significant increase in airway resistance and decrease in the difference between the alveolar and arterial oxygen pressures was observed. The effect of a nitrogen dioxide concentration of 9400 $\mu g/m^3$ (5.0 ppm) was not further enhanced by combination with ozone at a concentration of 200 $\mu g/m^3$ (0.1 ppm) or by combination with the same concentration of ozone and sulfur dioxide at 13 000 $\mu g/m^3$ (5.0 ppm), respectively. However, the recovery time was delayed in the last two experiments. Exposure to a combination of nitrogen dioxide at 100 $\mu g/m^3$ (0.05 ppm), ozone at 50 $\mu g/m^3$ (0.025 ppm), and sulfur dioxide at 260 $\mu g/m^3$ (0.10 ppm) for 2 h showed no effect on airway resistance or on the difference between the alveolar and arterial oxygen pressures. However, in these studies there was a dose-dependent increase in the sensibility of the bronchial tree to acetylcholine as compared with the control.

Nieding et al., (1973b) also investigated the acute effects of nitric oxide on lung function in man and found that although nitric oxide had an adverse effect on the human lung function, it was markedly less toxic than nitrogen dioxide.

6.2 Accidental and Industrial Exposures

In certain occupations, workers are intermittently exposed to high concentrations of oxides of nitrogen, particularly nitric oxide and nitrogen dioxide. These exposures occur in work that involves welding; in the industrial use of nitric acid compounds as in the production of sulfuric, picric, and chromic acids; in the manufacture of toluene, metallic nitrates and nitrocellulose (gunpowder); in the production of nitroglycerine and dynamite; and in mining and working in tunnels carrying motor vehicle traffic. Camiel & Berkan (1944) described a spectrum of pathological effects in the lung resulting from occupational exposure to nitrogen oxides; the effects varied from a mild inflammatory response in the mucosa of the tracheobronchial tree at low concen-

trations of oxides of nitrogen to bronchiolitis, bronchopneumonia, and acute pulmonary oedema at high exposures. Milne (1969) described a biphasic reaction to oxides of nitrogen in an industrial chemist engaged in manufacture of silver nitrate by mixing fuming nitric acid with silver. In reviewing the literature, Milne found that the biphasic response was quite typical of many reported cases of high industrial exposures to oxides of nitrogen. The biphasic reaction observed in acute industrial exposure to oxides of nitrogen provoked cough, dyspnoea, and a sense of strangulation immediately or shortly after exposure, apparent recovery over a latent period of 2-3 weeks, and finally the sudden onset of severe respiratory distress which terminated fatally or from which the worker apparently fully recovered. The early manifestations of the biphasic response were usually caused by acute bronchitis or pulmonary oedema, while the second and delayed phase was invariably due to bronchiolitis fibrosa obliterans. Lowry & Schuman (1956) described 4 cases of bronchiolitis fibrosa obliterans in farmers who had entered fresh-filled silos, in which high concentrations of nitrogen dioxide had built up. In each case, the farmer experienced cough and dyspnoea shortly after entering the silo. After several days, symptoms largely disappeared but were followed in 2 or 3 weeks by cough, malaise, weakness, dyspnoea, and fever. Chest roentogenograms showed multiple discrete nodules scattered in both lungs. Two of the patients died while the other 2 responded dramatically to high doses of steroids. The authors reported that nitrogen dioxide concentrations of 380–7500 mg/m^3 (200–4000 ppm) were measured in freshly filled experimental silos. Grayson (1956), reporting on 2 additional cases of nitrogen dioxide poisoning from silage gas, estimated that exposure to 560–940 mg/m^3 (300–500 ppm) is likely to result in fatal pulmonary oedema or asphyxia, 280–380 mg/m^3 (150–200 ppm) is associated with bronchiolitis fibrosa obliterans, 94–190 mg/m^3 (50–100 ppm) with reversible bronchiolitis and focal pneumonitis, and 47–140 mg/m^3 (25–75 ppm) with bronchitis or bronchopneumonia with complete recovery.

Müller (1969) reported the occurrence of prolonged cough, dyspnoea, and chronic bronchitis after acute exposure to oxides of nitrogen formed by underground blasting in mines. Kennedy (1972) examined 100 miners with a history of exposure to oxides of nitrogen fumes from underground shotfiring; he found that a new type of shell containing 50% ammonium nitrate and 34% magnesium nitrate had been introduced into British collieries in 1959 and was associated with an apparent marked increase in work absences due to chest illnesses. Analysis of the products of explosion revealed oxides of nitrogen levels of 88–167 ppm; conventional power shots produced oxides of nitrogen concentrations of 50 ppm or

more. Of the 100 miners, 84 had prolonged exposure to fumes from underground shotfiring, and most had residual volumes 150% higher than expected. Unfortunately, Kennedy's data are biased by the fact that the coal miners were referred to him because of the presence of respiratory disability and therefore were not representative of miners as a population; data concerning the smoking habits of the affected miners or the prevalence of impaired ventilatory status in miners not exposed to products of explosion were not obtained.

Unfortunately, there have been few follow-up studies of persons exposed intermittently or chronically to elevated concentrations of nitrogen dioxide. Gregory et al. (1969) performed a study on the mortality of survivors from the Cleveland Clinic fire of May, 1929. At that time, nitrocellulose was the basic material for X-ray film. Apparently the film storage area of the Cleveland Clinic was badly ventilated and the flammable gas given off by the film ignited resulting in the rapid formation of nitric oxide, nitrogen dioxide, carbon monoxide, and hydrogen cyanide. Within 2 hours, 97 persons died, and within the next 30 days another 26 died. The overall survival during the next 30 years, of clinic employees, firemen and policemen at the scene, and rescue workers was evaluated by Gregory et al. (1969) and compared with that of an unexposed group consisting of persons who were comparable in job and economic status but were definitely not at the scene of the disaster. None of the exposed groups showed any difference in survival suggesting no residual excess mortality due to acute exposure to the mixture of gases, which included an estimated nitric oxide concentration of 63 000 mg/m^3 (51 500 ppm). Clearly, the study suffers from lack of data on exposure of the various "exposed" groups, as well as from lack of more refined follow-up data on the survivors.

Mogi et al. (1968) and Yamazaki et al. (1969) evaluated pulmonary function in 475 railway workers in Japan employed in tunnels and repair sheds in which diesel exhaust fumes were concentrated. Although the best respiratory function was among employees who worked where the pollution level was lowest, no consistent gradient of pulmonary function was associated with areas of low, medium, and high concentrations of nitrogen dioxide.

Giguz (1968) found an 11–24% higher incidence of acute respiratory disease in 140 adolescents in the USSR engaged in vocational training in a nitrogen fertilizer manufacturing plant, than in 85 adolescents taking vocational training involving little or no contact with chemicals. The author states that average concentrations of ammonia and oxides of nitrogen in the fertilizer plant did not exceed the maximum permissible levels of the USSR but monitoring data are not presented in the report.

The possibility of other causal or contributory factors was not discussed.

Thus, the literature on industrial exposure to oxides of nitrogen provides little useful data on the chronic or acute effects of low level exposures. Follow-up studies of special occupational groups should be conducted in order to provide better data for occupational health standards for oxides of nitrogen.

6.3 Community Exposures

In comparison with the large number of epidemiological studies of populations exposed to sulfur oxides and particulate matter, there have been few investigations in which nitrogen dioxide was considered as the primary environmental factor in community exposure.

Prior to 1973, methods for measuring nitrogen dioxide in ambient air had been subject to a number of analytical and instrumental difficulties (Hauser & Shy, 1972), and few reliable data on community exposures were obtained until recent years. In addition, in the general community environment, nitrogen dioxide results from the high temperature combustion of fossil fuels and is nearly always found in combination with other fossil fuel combustion products such as sulfur dioxide, particulates, and hydrocarbons. Thus, there have been few opportunities to study populations in which observed health effects could be attributed mainly to nitrogen dioxide exposure. Epidemiological data concerning the health effects of nitric oxide are not available.

6.3.1 Effects on pulmonary function

Several epidemiological surveys of lung function in relation to community exposure to nitrogen dioxide have been reported. Shy et al. (1970a) observed slightly lower ventilatory function, adjusted for age, sex, and height, in 306, 7- and 8-year-old school children living in close proximity to a large industrial source of nitrogen dioxide. The results were of borderline significance, and elevated concentrations of sulfate and nitrate particulates were also reported. Speizer & Ferris (1973a) performed pulmonary function tests on 267 central city and suburban Boston policemen with different levels of exposure to automobile exhaust. In spite of differences in concentrations of nitrogen dioxide and sulfur dioxide, no differences were found in the results of any of the tests. Test results were standardized for age, height, and cigarette smoking habits. In a preliminary report, Speizer & Ferris (1976) suggested that the combination of smoking and automobile exhaust exposure accounted

for a significant decline in pulmonary diffusing capacity in a follow-up study of the Boston policemen 3 years after their first survey. Cohen et al. (1972) compared a variety of pulmonary function tests in 136 nonsmoking Seventh Day Adventists living in Los Angeles, where concentrations of nitrogen dioxide and oxidants were relatively high, with 207 members of the same religious affiliation living in San Diego, California, where levels were lower. No group differences in lung function were detected.

Kagawa & Toyama (1975) and Kagawa et al. (1976) studied the weekly variation in pulmonary function of 20, normal, 11-year-old school children in Tokyo in relation to variations in temperature and ambient concentrations of ozone, nitric oxide, nitrogen dioxide, hydrocarbons, sulfur dioxide and particulate matter. Students were tested from June 1972 to October 1973. Oxides of nitrogen were determined by the Saltzman method. Temperature was the factor most closely correlated with variations in specific airway conductance (negative correlation) and maximum expiratory flow rate ($\dot{V}max$) at 25% and 50% forced vital capacity (FVC) (positive correlation). Significant negative correlations were observed in sensitive children between ozone and specific airway conductance, and between nitrogen dioxide, nitric oxide, sulfur dioxide and particulate matter and $\dot{V}max$ at 25% or 50% FVC. During the high temperature season (April–October), nitrogen dioxide, sulfur dioxide, and particulate matter were significantly negatively correlated with both $\dot{V}max$ at 25% or 50% FVC and specific airway conductance. In one subject it was observed that $\dot{V}max$ at 50% FVC decreased steeply at nitrogen dioxide concentrations of 75 $\mu g/m^3$ (0.04 ppm) and above. However, the observed effect was not associated with nitrogen dioxide alone but with combined exposure to nitrogen dioxide, sulfur dioxide, particulate matter, and ozone. The range of hourly nitrogen dioxide concentrations at the time of the lung function test (1:00 pm), which was used for correlation during the period of study in the high temperature season was approximately 40 360 $\mu g/m^3$ (0.02–0.19 ppm).

6.3.2 Effects on the incidence of acute respiratory disease

Experimental animal studies described in section 5 established a causal relationship between nitrogen dioxide exposure and impaired resistance to respiratory infections. The mechanisms for this effect have been studied and include nitrogen dioxide-induced impairment of pulmonary clearance, antibody formation, interferon production, and bactericidal activity in lung tissue. Several epidemiological studies conducted in the USA and the USSR suggest that nitrogen dioxide

exposure may also impair resistance to respiratory infections in human populations, although the studies by no means incriminate nitrogen dioxide as the only responsible pollutant.

Lindberg (1960) reported a 17-fold excess in upper respiratory disease frequency in 1375 children living near a large superphosphate manufacturing plant in the USSR compared with 678 children living 10 km from the plant. Concentrations of oxides of nitrogen 34 times higher than the maximum allowable concentration of 2900 $\mu g/m^3$ (1.5 ppm) were found in the ambient air 500 m away from the plant together with sulfuric acid aerosol and fluorine. Excess respiratory disease could have been due to the mixture of pollutants or to other unmeasured substances emitted by the plant. In a similar study, Poljak (1968) reported that a population residing within 1 km of the chemical works in Šehelkovo, USSR, but not employed in the industry made 44% more visits to the health clinics for respiratory, visual, nervous system, and skin disorders than a population living more than 3 km away. A nitrogen dioxide concentration of 1600 $\mu g/m^3$ (0.85 ppm) combined with high concentrations of sulfur dioxide and sulfuric acid were reported 1000 m from the plant and this combination of pollutants may well have accounted for the observed respiratory effects.

Shy et al. (1970b) and Pearlman et al. (1971) evaluated the frequency of acute respiratory disease in children and their parents living near a large point source of nitrogen dioxide in Chattanooga, Tennessee. Three populations—one close to the source with a high nitrogen dioxide exposure, and 2 populations with low nitrogen dioxide exposure were included in the investigations. The incidence of acute respiratory disease was observed prospectively at 2-week intervals during the 1968-69 school year. After adjusting for group differences in family size and composition, the incidence of acute respiratory disease in the high exposure population was found to be 19% higher than in the 2 comparison groups (Shy et al., 1970b). Similarly in a retrospective study (Pearlman et al., 1971), the frequency of lower respiratory disease was found to be greater in 6- and 7-year-old children and in infants born between 1966 and 1969 in the area of high nitrogen dioxide exposure. Response was validated by physician and hospital records. Pollutant concentrations were monitored in the neighborhoods of each study population. However the original atmospheric measurements for nitrogen dioxide were based on the Jacobs-Hochheiser method, which has since been criticised for variable collection efficiency at different nitrogen dioxide concentrations (Hauser & Shy, 1972). Exposures of the population were therefore re-evaluated, using data obtained by the Saltzman method at an 11-station Chattanooga air monitoring network operated

Table 9.   Mean concentrations of pollutants ($\mu g/m^3$) in the Chattanooga studies [a]

Pollutant	High nitrogen dioxide exposure area			Comparison populations	
	School 1	School 2	School 3	Group A	Group B
nitrogen dioxide	282	150	150	113	56
sulfur dioxide	< 26	< 26	< 26	< 26	< 26
suspended sulfates	13.2	11.4	10.0	9.8	10.0
suspended nitrates	7.2	6.3	3.8	2.6	1.6
total suspended particulates	96	83	63	72	62

[a]Adapted from: Shy et al., 1970a; US Environmental Protection Agency 1976b.

by the US Army and the US Environmental Protection Agency for a period of 14 months immediately preceding the health studies (US Environmental Protection Agency, 1976b). The nitrogen dioxide data and data on other pollutants obtained during the study and presented in Table 9 show that the largest group differences in pollutant exposures were in the concentrations of nitrogen dioxide and suspended nitrates. However, it was known that the point source of nitrogen dioxide (a trinitrotoluene manufacturing plant) had experienced problems with sulfuric acid emissions into the atmosphere prior to the study, and these emissions, along with nitric acid fumes, nitrates and nitrogen dioxide, may have contributed to the observed excess in acute respiratory disease. As in all complex low level exposures, it is not possible to implicate one pollutant as the responsible agent for the excess disease reported in the Chattanooga studies.

Investigators from the US Environmental Protection Agency (1976b) compared the incidence of acute respiratory disease among housewives cooking with either gas or electric stoves. Because of high flame temperatures, gas stoves fix atmospheric nitrogen resulting in peak $\frac{1}{2}$-1h nitrogen dioxide concentrations of 940 $\mu g/m^3$ (0.5 ppm). Electric stoves do not operate at a temperature sufficiently high to form nitrogen dioxide. Housewives cooking with gas stoves did not show any evidence of increased respiratory disease and the results suggest that short-term intermittent exposures of 940 $\mu g/m^3$ or more do not appear to impair respiratory defence mechanisms in adult women.

Petr & Schmidt (1967) reported excess acute respiratory disease among children living near a large chemical complex, compared with children living in relatively clean towns. However, the authors did not provide data on concentrations of nitric oxide and nitrogen dioxide individually, and did not measure other pollutants such as sulfuric acid, sulfates, and total particulate matter which may have been produced by the chemical factories.

The epidemiological studies of acute respiratory disease in populations exposed for long periods to elevated nitrogen dioxide concentrations provide evidence that supports the animal data on nitrogen dioxide-induced impairment of resistance to respiratory infection. Failure to provide data on other pollutants present at the same time or on peak daily concentrations of nitrogen dioxide is a serious shortcoming in most of these (and other) epidemiological studies. It is difficult to determine whether a given concentration of nitrogen dioxide was responsible for the observed health effects, or whether one of the other pollutants, alone or in combination with nitrogen dioxide, was the causal agent. The health effects may have been due to prolonged exposure (associated with yearly averaging times) or to repeated small insults to the respiratory system caused by daily peak exposures of one or more hours duration. Judging by experimental animal data cited in section 5, intermittent peak exposures superimposed on longer periods of low-level exposure may play a dominant role in the development of impaired resistance to acute respiratory infection.

6.3.3 Effects on the prevalence of chronic respiratory disease

The experimental basis for the effect of nitrogen dioxide on the parenchymal tissue of the lung is well established and has been discussed in section 5. Few epidemiological studies concerning the relationship between chronic respiratory disease prevalence and population exposure to nitrogen dioxide have provided a consistent pattern of results.

Fujita and associates (1969) observed an increased prevalence of chronic bronchitis among 7800 post office employees in the Tokyo, Tsurumi, and Kawasaki areas surveyed on 2 occasions in 1962 and 1967. The authors attributed the doubling of bronchitis prevalence found in 1967, compared with 1962, to increasing atmospheric concentrations of sulfur dioxide and nitrogen dioxide. However, the authors' report provided inadequate documentation of air pollution concentrations, and the data which were presented suggested relatively high levels of sulfur dioxide (290 $\mu g/m^3$, 0.11 ppm) and low concentrations of nitrogen dioxide (75 $\mu g/m^3$, 0.04 ppm) in 1966.

An Expert Committee on Air Quality Criteria for Oxides of Nitrogen and Photochemical Oxidants, Japan (1972) reported the preliminary results of a 6-city survey of chronic bronchitis prevalence among 400 housewives living in each of the cities. Although the prevalence of disease by city was correlated with levels of nitrogen dioxide and nitric oxide, relatively high average concentrations of total suspended parti-

culates (350–500 μg/m³) and of sulfur dioxide (100–130 μg/m³, 0.04–0.05 ppm) were measured in the more polluted cities where the highest prevalence of chronic bronchitis was observed. On the other hand, nitrogen dioxide concentrations were relatively low in the more polluted cities, ranging from average values of 38 to 140 μg/m³ (0.02–0.077 ppm). It appears, therefore, that the differences in bronchitis prevalence were more likely to be associated with exposures to a particulate/sulfur oxides complex than to nitrogen dioxide.

As a follow-on to the Chattanooga studies reported earlier, Chapman et al. (1973) evaluated the prevalence of chronic bronchitis in 3500 adults who were parents of high school children residing in the 3 study areas of Chattanooga reported in Table 9. Although cigarette smoking and alveolar carbon monoxide concentrations obtained from end-expiratory breath samples showed significant correlations with the prevalence and severity of chronic respiratory disease, the study did not demonstrate an association between exposure to nitrogen dioxide and disease prevalence. Similarly, Cohen et al. (1972) failed to find a difference in the prevalence of chronic respiratory disease on comparing nonsmoking Seventh Day Adventists residing in Los Angeles and in San Diego, California. Mean nitrogen dioxide concentrations from 1963 to 1967 were 94 μg/m³ (0.05 ppm) in Los Angeles and 38 μg/m³ (0.02 ppm) in San Diego.

Speizer & Ferris (1973b) studied chronic bronchitis prevalence in 128 Boston policemen who patrolled congested business and shopping areas of central Boston with 140 suburban policemen who travelled in patrol cars in less congested suburban Boston communities. A slight but not statistically significant excess in chronic respiratory disease was found in nonsmokers and current smokers, but not in ex-smokers, who spent more time in heavy traffic. In central Boston, the mean nitrogen dioxide concentration was 100 μg/m³ (0.055 ppm) and the sulfur dioxide concentration was 91 μg/m³ (0.035 ppm). Suburban concentrations were 75 μg/m³ (0.04 ppm) and 26 μg/m³ (0.01 ppm) for nitrogen dioxide and sulfur dioxide respectively (Burgess et al., 1973).

Shimizu (1974) reported an increase in the prevalence of chronic bronchitis on comparing the results of 2 surveys of all residents over 40 years of age in selected districts of the Osaka Prefecture, Japan. The first survey was conducted from 1962 to 1966, and the second survey from 1970 to 1972. During the years between the 2 surveys, concentrations of sulfur oxides decreased mainly due to a marked decrease in the sulfur content of fuel oil. On the basis of air dispersion models and data on fuel consumption by stationary and mobile sources of oxides of nitrogen the concentration of oxides of nitrogen was calculated theoretically for each

square kilometre of the Osaka Prefecture, and oxides of nitrogen concentrations were estimated to have increased. However, no actual measurements of nitrogen dioxide were available in this study.

Based on surveys of chronic respiratory disease in 5 cities of the Chiba Prefecture, Japan, Yoshida et al. (1976) reported significant positive correlations between the prevalence of persistent cough and phlegm in adults, aged 40 and over, living within 2 kilometres of an air monitoring station, and the concentrations of sulfur dioxide and nitrogen dioxide. The authors expressed this association in the form of a multiple regression equation:

$$Y = 1.98 \, X_1 + 1.14 \, X_2 - 1.63$$

where Y = age, sex, and smoking adjusted prevalence of persistent cough and phlegm (%), X_1 = mean annual sulfur dioxide concentration (pphm) and X_2 = mean annual nitrogen dioxide concentration (pphm). In order to attain 3% of chronic brochitis prevalence rate, which is supposed to be a "natural" prevalence rate under the Japanese sulfur dioxide ambient air standard (0.04 ppm or 100 $\mu g/m^3$ daily mean, 0.018 ppm or 47 $\mu g/m^3$ annual mean), it was calculated that the annual nitrogen dioxide concentration should be below 17 $\mu g/m^3$ (0.009 ppm).

The epidemiological studies of chronic respiratory disease prevalence described earlier do not establish an association between disease prevalence and population exposures to nitrogen dioxide *per se*. In general, large differences in group exposure to nitrogen dioxide did not exist in these studies, thus diminishing the likelihood of finding a pollutant-related change. The Japanese studies are noteworthy in suggesting an increase in chronic respiratory disease prevalence over time periods when nitrogen dioxide exposures were estimated to increase. These results suggest the need for more longitudinal studies of populations exposed to changing concentrations of air pollutants. Such opportunities exist in rapidly developing urban areas, where the standard of living and related industrial and transportation activity are likely to change over a relatively short time.

6.4 Summary Tables

Table 10 is a summary of controlled human studies which provide a quantitative basis for evaluating health risks from exposure to nitrogen dioxide. Table 11 recapitulates epidemiological studies which tend to support evidence from animal experiments and controlled human studies, though they do not furnish quantitative information in establishing guidelines for health protection.

Table 10. Controlled human studies

I. Sensory effects

Nitrogen dioxide concentration		Length of exposure	Effects	Response [a]	Subjects	Reference
($\mu g/m^3$)	(ppm)					
790	0.42		odour perceived immediately after beginning of the exposure	8/8	8 healthy subjects	Henschler et al 1960
410	0.22		odour perceived immediately after beginning of the exposure	8/13	13 healthy subjects	Henschler et al 1960
230	0.12		odour perceived immediately after beginning of the exposure	3/9	9 healthy subjects	Henschler et al. 1960
230	0.12		odour perceived immediately after beginning of the exposure	"most" of the subjects	14 healthy subjects	Šalamberidze (1967)
200	0.11		odour perceived immediately after beginning of the exposure	26/28	28 healthy subjects	Feldman (1974)
0–51 000	0–27	54 min	no odour perception, when raising the concentration slowly within 54 min from 0 to 27 ppm; increase of relative humidity enhanced odour perception	0/6	6 healthy subjects	Henschler et al (1960)
140	0.074	5 and 25 min	decreased dark adaptation in all subjects (nasal breathing)	4/4	4 healthy subjects	Šalamberidze (1967)

[a] Response = number of subjects showing effects/total number of subjects

Table 10. Controlled human studies—*continued*

II. Effects on lung function

Nitrogen dioxide concentration		Length of exposure		Effects	Subjects	Reference
(µg/m³)	(ppm)	(number of days)	(h/day)			
9400	5	1	2	significant increase of R_{aw},[a] decrease of $AaDO_2$,[c] under intermittent light exercise	11 healthy subjects	Nieding et al. (1976)
9400	5	1	15 min	PAO_2[e] before, during and after exposure unchanged, but PaO_2[f] significantly decreased; $AaDO_2$ increased	14 chronic bronchitis patients	Nieding et al. (1973a)
9400	5	1	15 min	DL_{co}[d] significantly decreased	16 healthy subjects	Nieding et al. (1973a)
7500 9400	4 5	1	10 min	decrease in lung compliance with corresponding increases in expiratory and inspiratory flow resistance	5 healthy subjects	Abe (1967)
3000 3800	1.6 2.0	1	15 min	R_{aw} - increase	15 patients with chronic bronchitis	Nieding et al. (1971)
1300–3800	0.7–2.0	1	10 min	increase in inspiratory and expiratory flow resistance	10 healthy subjects	Suzuki & Ishikawa (1965)
190	0.1	1	1	a slight but significant increase in initial SR_{aw}[b] and enhancement of bronchoconstrictor effect of carbachol in 13 subjects	20 asthmatics	Orenek et al. (1976)
100 in combination with 50 ozone and 260 sulfur dioxide	0.05 in combination with 0.025 ozone and 0.10 sulfur dioxide	1	2	no effect on R_{aw} and $AaDO_2$; sensitivity of the bronchial tree to acetylcholine increased compared with that before exposure to the pollutants	11 healthy subjects	Nieding et al. (1976)

[a] R_{aw} = Airway resistance
[b] SR_{aw} = Specific airway resistance, i.e. the product of airway resistance and thoracic gas volume
[c] $AaDO_2$ = Alveolar to arterial oxygen pressure difference
[d] DL_{co} = Diffusing capacity of the lung for carbon monoxide
[e] PAO_2 = Alveolar partial pressures of oxygen
[f] PaO_2 = Arterial partial pressures of oxygen

Table 11. Epidemiological studies of community exposure

I. Pulmonary function

Concentrations and population	Averaging time	Effect and/or response	Reference
Pulmonary function tests on twenty normal 11-year-old school children were made once or twice a week for 17 months. The concentrations of nitrogen dioxide in the higher temperature season at the time of measurement ranged from approximately 40–360 µg/m³ (0.02–0.19 ppm).	1 h	association with decrease in specific airway conductance and Vmax at 50% FVC during the high temperature season, in one subject. Vmax at 50% FVC decreased steeply at nitrogen dioxide levels of approximately 75 µg/m³ (0.04 ppm); the observed effect is not associated with nitrogen dioxide alone, but with combined exposure to nitrogen dioxide, sulfur dioxide, particulates and ozone.	Kagawa et al. (1976)

II. Acute respiratory disease

Concentrations		Averaging time	Effect and/or response	Reference
Exposed	Control			
150–282 µg/m³ (0.08–0.15 ppm) nitrogen dioxide with 4–7 µg/m³ nitrates, 10–13 µg/m³ sulfates, <26 µg/m³ (<0.01 ppm) sulfur dioxide	56–113µg/m³ (0.03–0.06 ppm) nitrogen dioxide with 2–3 µg/m³ nitrates, 10 µg/m³ sulfates, <26 µg/m³ (<0.01 ppm) sulfur dioxide	1 year	increased incidence of acute respiratory disease in school children and parents in Chattanooga.	Shy et al. (1970a, 1970b)
63–96 µg/m³ particulates; exposure to sulfuric acid and nitric acid fumes also present but not measured.	62–72 µg/m³ particulates		increased incidence of lower respiratory disease in Chattanooga infants and school children	Pearlman et al. (1971)
≥940 µg/m³ (≥0.50 ppm) nitrogen dioxide ½–1 h peak indoor concentration	<940 µg/m³ (<0.50 ppm) nitrogen dioxide	1 h	no evidence of increased acute respiratory disease in housewives cooking with gas stoves compared with those using electric stoves.	US Environmental Protection Agency (1976b)

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Table 11. Epidemiological studies of community exposure—*continued*

III. Chronic respiratory disease

Concentrations		Averaging time	Effect	Reference
Exposed	Control			
100 μg/m³ (0.055 ppm) nitrogen dioxide with 91 μg/m³ (0.035 ppm) sulfur dioxide	75 μg/m³ (0.04 ppm) nitrogen dioxide with 26 μg/m³ (0.01 ppm) sulfur dioxide	1 year	no significant increase in chronic respiratory symptoms among central city traffic police officers in Boston.	Speizer & Ferris (1973b)
94 μg/m³ (0.05 ppm) nitrogen dioxide with 26 μg/m³ (0.01 ppm) sulfur dioxide, 120 μg/m³ particulates, 280 μg/m³ (0.14 ppm) oxidants (mean of daily 1-h maxima)	43 μg/m³ (0.023 ppm) nitrogen dioxide with 26 μg/m³ (0.01 ppm) sulfur dioxide, 78 μg/m³ (0.074 ppm) particulates, 150 μg/m³ (0.074 ppm) oxidants (mean of daily 1-h maxima)	1 year	no effect on prevalence of chronic respiratory symptoms or on lung functions of nonsmoking subjects living in Southern California.	Cohen et al. (1972)

7. EVALUATION OF HEALTH RISKS FROM EXPOSURE TO OXIDES OF NITROGEN

It is well established that respiratory disease is an important cause of disability and death. There is also considerable evidence that some of these diseases are associated with the inhalation of polluted air. Most of these associations have been established with regard to the presence in the ambient air of sulfur dioxide, particulate matter, and/or smoke (World Health Organization, 1972). Oxides of nitrogen as well as some other pollutants were not considered in the studies on sulfur oxides and suspended particulates although it is likely that they were present. It is possible that nitrogen dioxide could play a role in causing respiratory disease but, to date, only a limited number of epidemiological investigations have been carried out with regard to the effects on human health of this pollutant. There is, however, a considerable amount of data derived from experimental animal studies and controlled studies on human volunteers showing a high biological activity of nitrogen dioxide even at low concentrations. These data are useful as bases for assessing the toxic effects of nitrogen dioxide and for establishing guidelines for exposure limits for the protection of public health.

At present, there is no evidence that nitric oxide concentrations typically observed in the ambient air have a significant biological effect. The Task Group did not, therefore, develop guidelines for nitric oxide exposure limits for the protection of public health.

7.1 Exposure Levels

Exposure of human populations to nitrogen dioxide varies widely both with respect to time and place. In rural areas, far from man-made sources, nitrogen dioxide concentrations have been estimated at 5 $\mu g/m^3$ (0.0025 ppm), while in most major cities annual means of 20–90 $\mu g/m^3$ (0.01–0.05 ppm) have been recorded. In most of these cities the maximum 24-h means range from 130 to 400 $\mu g/m^3$ (0.07–0.21 ppm). Peak concentrations may be substantially higher. In some of the larger urban areas, maximum 1-h concentrations in excess of 800 $\mu g/m^3$ (0.43 ppm) have been measured. The available data indicate that in most situations the maximum 24-h mean in a given year is 2–5 times higher than the annual mean for that location. The 1-h maximum values are about 5–10 times higher than the recorded annual means. These peak concentrations generally occur on clear days twice daily, in the morning and evening hours.

Normally, nitrogen dioxide is accompanied by many other air pollutants such as particulate matter, carbon monoxide, sulfur dioxide, ozone etc. and this is of special concern from an epidemiological point of view, since the effects on human health may well be additive or even synergistic.

Another aspect which must be considered is that a certain portion of the population is also exposed, though intermittently, to extremely high concentrations of nitrogen dioxide in their working or home environment or due to the inhalation of tobacco smoke. Cigarette smoke may contain nitrogen dioxide concentrations as high as 226 mg/m^3 (120 ppm). Directly above gas stoves, nitrogen dioxide concentrations may reach levels as high as 2000 µg/m^3 (1.1 ppm).

Current trends indicate that emissions of oxides of nitrogen will continue to increase, primarily because of increased use of fossil fuels both in stationary sources and transportation.

7.2 Experimental Animal Studies

The toxic effects of nitrogen dioxide have been studied extensively in a wide variety of experimental animal models. Analysis of the available data clearly indicates that a number of factors can influence the host's response to this air pollutant. The species of animals tested, the duration, concentration, and mode of exposure, and the pre-existence of disease can modify the expected response to nitrogen dioxide. A summary of selected studies is given in Table 8.

The primary target of nitrogen dioxide is the respiratory system. A variety of effects have been measured which can be related to the concentration and time of the nitrogen dioxide exposure. Effects measured include changes in pulmonary function, morphological changes, depression of host defence mechanisms, oedema and, at high concentrations, death. In addition a number of systemic or extra-pulmonary responses have also been observed, i.e. decrease in growth rate; alteration in immunological response; polycythemia and leuco-cytosis; changes in reproductive function; delay in conditioned reflexes of the central nervous system; and depression in physical activity. The lowest concentration at which adverse effects on pulmonary function were found was 1500 µg/m^3 (0.8 ppm). At this level the respiratory rates of rats remained elevated throughout life.

Continuous exposure of mice, rats, or rabbits to concentrations of 470–1900 µg/m^3 (0.25–1.0 ppm) produced a number of morphological changes in the respiratory system. Structural changes in lung collagen,

alveolar distension, shortening of ciliated epithelial cells, and adenomatous proliferation of bronchial and bronchiolar epithelium have been observed after exposures of 30 days or less. With long-term exposure of various animal species (rat, rabbit, monkey, guineapig) to higher concentrations (3800–47 000 $\mu g/m^3$, 2–25 ppm), the above effects became more pronounced and changes in respiration rate, tidal volume, immunological and biochemical parameters became noticeable.

Exposure to nitrogen dioxide has been shown to increase the susceptibility of the host to respiratory infections. This effect is clearly dose-related and has been shown with short-term, continuous, and intermittent exposures. When mice were exposed for 90 days to a nitrogen dioxide concentration as low as 940 $\mu g/m^3$ (0.5 ppm) and then immediately given a laboratory-induced infection, a significant increase in mortality rate was observed. Nitrogen dioxide can also enhance the risk of respiratory infection in other animal species (hamster, squirrel monkey) although considerably higher concentrations are required (e.g. 1–2 months treatment with 9400 $\mu g/m^3$–19 mg/m^3 (5–10 ppm).

7.3 Controlled Studies in Man

Controlled human studies which can be used for evaluating health effects are limited to short-term exposures. It can be seen from Table 10 that functional changes of the lung in healthy human subjects such as an increase in air way resistance begin after 10 min of inhalation of nitrogen dioxide concentrations of 1300 $\mu g/m^3$ (0.7 ppm) or more. Recently it was shown that the reaction to inhalation challenge with a bronchoconstrictor (carbachol) in asthmatic subjects increased after exposure to a nitrogen dioxide concentration of 190 $\mu g/m^3$ (0.1 ppm) for 1 h. A similar reaction was shown in healthy subjects exposed to a combination of nitrogen dioxide at 100 $\mu g/m^3$ (0.05 ppm), ozone at 50 $\mu g/m^3$ (0.025 ppm), and sulfur dioxide at 260 $\mu g/m^3$ (0.10 ppm) for 2 h. These reactions might be of importance especially in subjects with respiratory disease when gaseous pollutants act in combination with inhaled particles such as pollen, spores, suspended particulate matter, or dust. The olfactory threshold for nitrogen dioxide and the level at which changes in dark adaptation occurred were both about 200 $\mu g/m^3$ (0.11 ppm).

7.4 Effects of Accidental and Industrial Exposures

Inadvertent and accidental exposure of human subjects to high concentrations of nitrogen dioxide has occurred among welders, farmers

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working in freshly filled silos, miners using explosive chemicals and in other occupations. Exposure levels have not been precisely documented in these situations, but severe acute and delayed effects were experienced in the form of pneumonia, bronchiolitis and sometimes pulmonary oedema. From these studies, it has been estimated that short-term exposures of 1 h or less to nitrogen dioxide concentrations of 47–140 mg/m^3 (25 75 ppm) can cause pneumonia and bronchitis, while exposure to 560 940 mg/m^3 (300 500 ppm) may cause fatal, pulmonary oedema or asphyxia.

In general, acute or chronic effects of low level industrial exposure to nitrogen dioxide have not been systematically evaluated.

7.5 Effects of Community Exposures

In comparison with the large body of data on populations exposed to sulfur oxides and particulate matter, there are few epidemiological studies in which nitrogen dioxide has been considered to be the primary environmental factor in community exposures. The epidemiological studies described in section 6.3 demonstrate increased risk of acute respiratory disease and diminished lung function particularly among school children exposed to community air containing nitrogen dioxide, sulfur oxides, particulate matter, and, in some cases, photo-chemical oxidants. It is difficult to determine whether a given level of nitrogen dioxide was responsible for the observed health effects, or whether one of the other pollutants, alone or in combination with nitrogen dioxide was the causal agent. Furthermore, the health effects may have been due to chronic exposures or to repeated small insults to the respiratory system associated with exposures to daily peak concentrations. Judged by experimental animal data, intermittent peak values superimposed on longer periods of low level exposure may play a dominant role in the development of impaired resistance to acute respiratory infections.

The Task Group concluded, therefore, that the results of reported epidemiological studies cannot themselves provide a quantitative basis for evaluating health risks from exposure to nitrogen dioxide. In particular, the Task Group agreed that a specific concentration of nitrogen dioxide for a given averaging time could not be conclusively associated with the health effects observed in various epidemiological studies. The significance of the reported studies is that they support evidence from animal experiments and controlled human studies of increased risk of acute respiratory infections and altered lung function.

7.6 Evaluation of Health Risks

As has already been mentioned, there are not enough epidemiological data related to occupational or community exposures to serve as a basis for developing reliable air quality guides for nitrogen dioxide and for quantitative risk evaluation. However, the existing data do not contradict the findings that pulmonary effects are related to nitrogen dioxide exposure.

Thus, in an attempt to develop recommendations for guidelines on exposure limits consistent with the protection of human health, the Task Group had to rely mainly upon data from animal experiments and controlled human studies. The Group considered as adverse effects not only the morphological and other changes caused by higher nitrogen dioxide concentrations but also the effects on the respiratory system induced by lower concentrations. These changes include increased airway resistance, increased sensitivity to bronchoconstrictors, and enhanced susceptibility to respiratory infections. Although some of these effects were reversible, the Task Group's opinion was that such effects should be prevented. The Task Group estimated that adverse effects on the respiratory system of test animals might arise with short-term as well as long-term exposure to nitrogen dioxide at concentrations beginning from approximately 940 $\mu g/m^3$ (0.5 ppm). Adverse effects in man have occurred at approximately the same concentrations of nitrogen dioxide. Under controlled conditions human subjects exposed to nitrogen dioxide concentrations of 1300–3800 $\mu g/m^3$ (0.7 2.0 ppm) for 10 min exhibited increased airway resistance. Furthermore exposure to a nitrogen dioxide concentration of 190 $\mu g/m^3$ (0.1 ppm) for 1 h enhanced the bronchoconstrictor effect of a chemical aerosol (carbachol) in asthmatics.

A WHO Expert Committee in 1972 examined available information on some air pollutants including nitrogen dioxide. The biological activity of nitrogen dioxide in animals as well as plants was recognized but the Expert Committee believed that there was insufficient information upon which to base specific air quality guides in the absence of conclusive epidemiological data (World Health Organization, 1972).

The present Group felt it appropriate and prudent not to wait for more conclusive epidemiological evidence but to use available controlled study data from animals and human subjects in an attempt to develop guidelines for exposure limits consistent with the protection of public health. Such an approach seemed even more reasonable since results of epidemiological studies tended to support these data.

The Task Group selected the nitrogen dioxide level of 940 $\mu g/m^3$ (0.5 ppm) as an estimate of the lowest observed effect-level for short-term

exposures, because, at this concentration, effects had been shown in many controlled studies on animals and man. The Task Group was aware that one controlled human study showed an adverse effect at a lower concentration of 190 $\mu g/m^3$ (0.1 ppm) in asthmatics. This study needs confirmation and the Task Group agreed that at present the lowest adverse effect level for highly sensitive human subjects is not known and needs to be assessed. In view of the uncertainty concerning the lowest adverse effect level and the high biological activity of nitrogen dioxide, the Task Group concluded that a considerable safety factor was required. The difference between the approximate lowest observed effect level of 940 $\mu g/m^3$ (0.5 ppm) for 1 h and background concentrations of about 5 $\mu g/m^3$ (0.0025 ppm) would allow no more than a maximum safety factor of 200. The maximum safety factor is, in fact, reduced to a value of 20, since maximum hourly nitrogen dioxide concentrations in small towns and villages remote from pollution sources may reach 50 $\mu g/m^3$ (0.025 ppm). In larger cities, maximum hourly values may reach 470 $\mu g/m^3$ (0.25 ppm) or more. At approximately these concentrations some effects have been shown in a few controlled studies on man and animals. The Group considered this highly unsatisfactory, particularly in view of the fact that there is reason to believe that, if effective measures are not taken, concentrations of oxides of nitrogen in urban communities will rise due to increased use of fossil fuels.

Any safety factor must be arbitrary but, obviously, it should be sufficient to protect populations living in large urban communities. Taking into consideration all available information, the Task Group decided to propose a minimum safety factor of 3–5 for short-term exposure to nitrogen dioxide, and agreed that an exposure limit consistent with the protection of public health might be provided by a nitrogen dioxide concentration of 190 to 320 $\mu g/m^3$ (0.10 0.17 ppm) for a maximum 1-h exposure. This 1-h exposure should not be exceeded more than once per month.

Evidence on the interaction of nitrogen dioxide with other co-existing biologically active air pollutants may well suggest the need for larger safety factors and therefore lower maximum permissible exposure levels. Even now, there may be a need to increase the safety factor in order to protect the highly sensitive portion of the population.

In its evaluation of health risks, the Task Group believed that the biomedical effects of long-term exposure to nitrogen dioxide in man had not been ascertained to the extent that a recommendation for the protection of public health could be made, and therefore did not propose an exposure limit pertaining to long-term averaging times.

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