criteria for a recommended standard....

OCCUPATIONAL EXPOSURE TO OXIDES OF NITROGEN

(NITROGEN DIOXIDE AND NITRIC OXIDE)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Center for Disease Control
National Institute for Occupational Safety and Health

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PREFACE

The Occupational Safety and Health Act of 1970 emphasizes the need for standards to protect the health and safety of workers exposed to an ever-increasing number of potential hazards at their workplace. The National Institute for Occupational Safety and Health has projected a formal system of research, with priorities determined on the basis of specified indices, to provide relevant data from which valid criteria for effective standards can be derived. Recommended standards for occupational exposure, which are the result of this work, are based on the health effects of exposure. The Secretary of Labor will weigh these recommendations along with other considerations such as feasibility and means of implementation in developing regulatory standards.

It is intended to present successive reports as research and epidemiologic studies are completed and as sampling and analytical methods are developed. Criteria and standards will be reviewed periodically to ensure continuing protection of the worker.

I am pleased to acknowledge the contributions to this report on the oxides of nitrogen (nitrogen dioxide and nitric oxide) by members of my staff and the valuable constructive comments by the Review Consultants on the Oxides of Nitrogen, by the ad hoc committees of the Society of Toxicology and the American Medical Association, by Robert B. O'Connor, M.D., NIOSH consultant in occupational medicine, and by William H. Revoir, Jr., on respiratory protection. The NIOSH recommendations for standards are not necessarily a consensus of all the consultants and professional societies

that reviewed this criteria document on oxides of nitrogen. Lists of the NIOSH Review Committee members and of the Review Consultants appear on the following pages.

John F. Finklea, M.D.
Director, National Institute for
Occupational Safety and Health

The Office of Research and Standards Development, National Institute for Occupational Safety and Health, had primary responsibility for development of the criteria and the recommended standard for the oxides of nitrogen (nitrogen dioxide and nitric oxide). Tabershaw-Cooper Associates, Inc., developed the basic information for consideration by NIOSH staff and consultants under contract No. HSM 99-73-26. B. Thomas Scheib, served as criteria manager and Douglas L. Smith, Ph.D. had NIOSH program responsibility for development of the document.

REVIEW COMMITTEES
NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH

Nitrogen Dioxide

Jack Butler, M.D.
Acting Associate Director,
Washington Operations

Marilyn K. Hutchison, M.D.
Acting Director, Division of
Occupational Health Programs

Richard A. Lemen
Division of Field Studies and
Clinical Investigations

Jack E. McCracken, Ph.D.
Office of Research and
Standards Development

Frank L. Mitchell, D.O.
Office of Research and
Standards Development

P. G. Rentos, Ph.D.
Office of Extramural Activities

William D. Wagner
Division of Laboratories and
Criteria Development

Department of Labor Liaison:
Ching-tsen Bien
Office of Standards

Nitric Oxide

John M. Bryant
Deputy Director, Division of
Laboratories and Criteria
Development

Paul E. Caplan
Deputy Director, Division of
Technical Services

Elliot S. Harris, Ph.D.
Director, Division of Laboratories
and Criteria Development

Marilyn K. Hutchison, M.D.
Acting Director, Division of
Occupational Health Programs

Frank L. Mitchell, D.O.
Office of Research and
Standards Development

Department of Labor Liaison:
Ching-tsen Bien
Office of Standards

NIOSH REVIEW CONSULTANTS ON THE OXIDES OF NITROGEN
(NITROGEN DIOXIDE AND NITRIC OXIDE)

Richard S. Brief
Acting Director, Industrial Hygiene
Medical Department, Research and
Environmental Health Division
Exxon Corporation
Linden, New Jersey 07036

Hervey B. Elkins, Ph.D.
Consultant, Industrial Toxicology and
Industrial Hygiene Chemistry
303 Mill Street
Belmont, Massachusetts 02178

Jon M. Heuss
Environmental Science Department
Research Laboratories, General Motors Technical Center
General Motors Corporation
Warren, Michigan 48090

Jeanne M. Stellman, Ph.D.
Consultant for Health and Safety
Oil, Chemical and Atomic Workers
International Union
Denver, Colorado 80201

Carl Zenz, M.D.
Medical Director
Allis-Chalmers Manufacturing Company
Milwaukee, Wisconsin 53201

CRITERIA DOCUMENT: RECOMMENDATIONS FOR OCCUPATIONAL EXPOSURE
STANDARDS FOR THE OXIDES OF NITROGEN
(NITROGEN DIOXIDE AND NITRIC OXIDE)

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I. RECOMMENDATIONS FOR A STANDARD FOR THE OXIDES OF NITROGEN (NITROGEN DIOXIDE AND NITRIC OXIDE)

The National Institute for Occupational Safety and Health (NIOSH) recommends that employee exposure to the oxides of nitrogen (nitrogen dioxide and nitric oxide) in the workplace be controlled by adherence to the following sections. The standard is designed to protect the health and safety of workers for up to a 10-hour workday, 40-hour workweek over a working lifetime; compliance with the standard is measurable by techniques that are valid, reproducible, and available to industry and government agencies. Sufficient technology exists to permit compliance with the recommended standard. The criteria and standard will be subject to review and revision as necessary.

For the purpose of this standard, "oxides of nitrogen" refers to nitric oxide and nitrogen dioxide. Since nitrogen dioxide in the working environment results, at least in part, from oxidation of nitric oxide, occupational exposures are customarily to mixtures of these gases rather than to either gas alone. "Occupational exposure to the oxides of nitrogen" is defined as exposure to the oxides of nitrogen equal to or above one-half the recommended workroom limit. Adherence only to sections 3, 4(a)(1)(B), 4(a)(1)(C), 4(a)(2)(G), 4(a)(2)(L), 4(a)(2)(M), 4(b)(2), 5, 6(d), 6(e), 6(f), 7(a)(2), 7(a)(3), 7(b)(6), and 7(b)(7) is required when workplace environmental concentrations of oxides of nitrogen are not greater than one-half of the recommended workplace environmental limit. Procedures for identification of exposure areas can be accomplished by determinations using sampling and analytical methods described in

Appendices I and II, or III or by any method shown to be equivalent in accuracy, precision, and sensitivity to the methods specified.

Section 1 - Environmental (Workplace Air)

(a) Nitrogen Dioxide (NO2)

Occupational exposure to nitrogen dioxide shall be controlled so that workers are not exposed to nitrogen dioxide at greater than a ceiling concentration of 1 ppm by volume (1.8 mg/cu m) as determined by a sampling time of 15 minutes.

(b) Nitric Oxide (NO)

Occupational exposure to nitric oxide shall be controlled so that workers are not exposed to nitric oxide at a concentration greater than 25 ppm of air (30 mg/cu m) determined as a time-weighted average (TWA) exposure for up to a 10-hour workday, 40-hour workweek.

(c) Sampling and Analysis

Procedures for collection and analysis of environmental samples shall be as provided for in Appendices I and II, or by any method shown to be equivalent in precision, accuracy, and sensitivity to the methods specified. Since nitrogen dioxide rarely exists independent of nitric oxide in the workplace air, a sufficient number of samples shall be taken to determine a ceiling concentration for nitrogen dioxide and a TWA for nitric oxide in order to characterize the worker's exposure to the oxides of nitrogen.

Section 2 - Medical

Medical surveillance with particular emphasis on the respiratory system shall be made available as specified below for all workers subject

to "exposure to the oxides of nitrogen."

(a) Preplacement and periodic medical examinations shall be made available to workers occupationally exposed to the oxides of nitrogen. These periodic examinations shall be administered annually or as otherwise indicated by the responsible physician, and shall include as a minimum:

(1) Comprehensive or interim medical and work histories.

(2) Pulmonary function tests including auscultation of the chest, measurement of Forced Vital Capacity (FVC), and Forced Expiratory Volume in the first second (FEV 1).

(3) A judgment of the worker's physical ability to use negative or positive pressure respirators as defined in 29 CFR 1910.134.

In addition to the above, a 14" x 17" posterior-anterior chest X-ray shall be included in the preplacement medical examination. Additional evaluations of pulmonary function, such as tests of pulmonary compliance and diffusion studies, may be found useful in the medical surveillance of workers exposed to nitrogen oxides.

(b) Initial examinations for presently employed workers shall be offered within 6 months of the promulgation of a standard incorporating these recommendations and annually thereafter.

(c) Medical records shall be maintained for persons employed in work involving exposure to the oxides of nitrogen and shall include information on all required medical examinations. X-rays and all pertinent medical records with supporting documents shall be maintained at least 20 years after the individual's employment is terminated. These records shall be available to the medical representatives of the Secretary of Health, Education, and Welfare, of the Secretary of Labor, of the employee or

former employee, and of the employer.

Section 3 - Labeling (Posting)

Areas where the oxides of nitrogen may be reasonably expected to be present, even occasionally, shall be posted with a clearly legible sign reading:

NITROGEN OXIDES

WARNING

Excessive exposure to these gases is hazardous to health.
Adequate ventilation must be provided.

This sign shall be printed in English and in the predominant language of non-English-speaking workers. All employees shall be informed of the hazardous areas with special instruction given to illiterate workers.

Section 4 - Personal Protective Equipment

Requirements for personal protective equipment shall be as approved under provisions of 29 CFR 1910.134.

(a) Respiratory Protection

(1) Engineering controls shall be used wherever feasible to maintain the concentrations of oxides of nitrogen at or below the prescribed environmental limits listed in paragraphs (a) and (b) of Section 1. While engineering controls are being installed and tested, appropriate respirators shall be provided by employers and used by employees pursuant to the following requirements:

(A) Respirators shall be used for routine operations which result in continuous or frequent exposure to oxides of nitrogen in air at concentrations exceeding prescribed limits only while engineering

controls are being installed and tested.

(B) Respirators shall be used for nonroutine operations of maintenance and repair which result in brief exposure to oxides of nitrogen at concentrations in air exceeding prescribed limits.

(C) Respirators shall be used during emergencies when the concentrations of oxides of nitrogen in air may exceed prescribed limits.

(2) When a respirator is permitted by paragraph (a) (1) of this Section, it shall be selected and used pursuant to the following requirements:

(A) For the purpose of selecting the respirator to be used by employees, the employer shall measure the concentrations of nitrogen dioxide and nitric oxide in the workplace air initially and thereafter whenever control, process, operation, worksite, or climate changes occur which may likely affect the concentrations of oxides of nitrogen. This does not apply during emergencies and during firefighting.

(B) The employer shall ensure that no employee is exposed at concentrations of oxides of nitrogen in excess of prescribed limits because of improper respirator selection, fit, use, or maintenance.

(C) A respiratory protection program meeting the requirements of 29 CFR 1910.134 and 30 CFR 11 shall be established and carried out by the employer.

(D) The employer shall provide employees with respirators in accordance with Table I-1 and shall ensure that employees use the respirators provided in a proper manner.

(E) Respirators described in Table I-1 shall be

those approved under the provisions of 30 CFR 11 as amended.

(F) Respirators specified in Table I-1 for use in atmospheres of higher concentrations of oxides of nitrogen may be permitted for use in atmospheres which contain lower concentrations.

(G) Wherever bulk nitrogen dioxide or nitric oxide is stored or is introduced into an operation or process from a source under pressure, the employer shall store emergency and escape-type respirators so that they are readily accessible to each employee.

(H) The employer shall assign an employee to a job which requires the wearing of a respirator for protection against oxides of nitrogen only with a medical evaluation of the employee's physical ability to safely perform his or her duties while wearing a respirator.

(I) The employer shall instruct the employee in how to don the respirator and how to check its fit and operation.

(J) The employer shall provide the employee with instructions in the proper wearing of the respirator.

(K) The employer shall instruct the employee to use the respirator provided in accordance with instructions and training received, to test the fit of the respirator before entering an atmosphere contaminated with oxides of nitrogen, to guard against damage to the respirator, and to report any malfunction of the respirator to his or her supervisor.

(L) The employer shall instruct the employee in how to recognize and handle emergency situations.

(M) The employer shall establish and carry out a program of cleaning, sanitizing, inspecting, maintaining, repairing, and

storing of respirators to ensure that employees are provided with respirators that are in good operating condition, and shall instruct employees in day-to-day maintenance of respirators.

(N) The employer shall periodically monitor the use of respirators to ensure that the proper type of respirator is being worn in a satisfactory manner.

(O) The employer shall periodically evaluate the effectiveness of the respiratory protection program and eliminate any deficiencies.

TABLE I-1

RESPIRATORS FOR PROTECTION AGAINST INHALATION OF NITROGEN DIOXIDE AND NITRIC OXIDE*

Maximum Use Concentration	Required Respirator
Less than or equal to 50 ppm of NO2 or 1250 ppm TWA of NO	(1) Full facepiece chemical cartridge respirator and NO2 cartridges (2) A gas mask with a front- or back-mounted NO2 canister (3) Any supplied-air respirator with a full facepiece, helmet, or hood (4) Any self-contained breathing apparatus with a full facepiece
Less than or equal to 100 ppm of NO2 or 1250 ppm TWA of NO	(1) Type C supplied-air respirator operated in demand mode (negative pressure) with full facepiece (2) Self-contained breathing apparatus operated in demand mode (negative pressure) with full facepiece

TABLE I-1 (CONTINUED)

RESPIRATORS FOR PROTECTION AGAINST INHALATION OF NITROGEN DIOXIDE AND NITRIC OXIDE*

Maximum Use Concentration	Required Respirator
Less than or equal to 200 ppm of NO2 or 1250 ppm TWA of NO	(1) Type C supplied-air respirator operated in continuous-flow mode (positive pressure) with full facepiece, helmet, or hood (2) Type C supplied-air respirator operated in pressure-demand mode (positive pressure) with full facepiece
Unknown or greater than 200 ppm of NO2 or 1250 ppm TWA of NO	(1) Combination Type C supplied-air respirator operated in continuous-flow mode (positive pressure) or pressure-demand mode (positive pressure) and auxiliary self-contained breathing air supply operated in pressure-demand mode (positive pressure) with full facepiece (2) Self-contained breathing apparatus operated in pressure-demand mode (positive pressure) with full facepiece
Emergency, Entry (No concentration limit)	(1) Combination Type C supplied air respirator operated in continuous-flow mode (positive pressure) or pressure-demand mode (positive pressure) and auxiliary self-contained breathing air supply operated in pressure-demand mode (positive pressure) with full facepiece (2) Self-contained breathing apparatus operated in pressure-demand mode (positive pressure) with full facepiece

TABLE I-1 (CONTINUED)

RESPIRATORS FOR PROTECTION AGAINST INHALATION OF NITROGEN DIOXIDE AND NITRIC OXIDE*

Maximum Use Concentration	Required Respirator
Emergency, Escape (No concentration limit)	(1) Self-contained breathing apparatus operated in pressure-demand mode (positive pressure) with full facepiece
Firefighting	(1) Self-contained breathing apparatus operated in pressure-demand mode (positive pressure) with full facepiece

*Respirators equipped with a quarter-mask or half-mask facepiece are not recommended because eye irritation may become significant at low concentrations of nitrogen dioxide in air.

(b) Eye and Face Protection

(1) Eye and face protection shall be achieved by adherence to the requirements of 29 CFR 1910.133.

(2) Chemical safety goggles -- cup-type or rubber-framed goggles, equipped with approved impact-resistant glass or plastic lenses, shall be worn whenever there is danger of eye contact, such as working with pipelines, valves, etc, which might leak and spurt liquid nitrogen dioxide or nitric oxide.

(3) Spectacle-type safety goggles -- metal- or plastic-rim safety spectacles with unperforated side shields, or suitable all-plastic safety goggles may be used where continuous eye protection is desirable.

(4) Face shield -- plastic shields with forehead protection should be worn in addition to goggles.

Section 5 - Informing Employees of Hazards from Oxides of Nitrogen

(a) Workers initially assigned or reassigned to jobs in which the concentrations of the oxides of nitrogen exceed the levels defined for occupational exposure or who will work in areas required to be posted in accordance with Section 3 shall be informed of hazards, relevant symptoms including information on the onset and stages of illness, appropriate emergency procedures, and proper conditions and precautions for safe use, and shall be instructed as to the availability of such information which shall be kept on file, including that prescribed in (c) below. This information shall be accessible to the worker at each place of employment where the oxides of nitrogen are involved in unit processes and operations or evolved as byproducts or contaminants from operations or processes.

(b) A continuing educational program shall be instituted to ensure that all workers have current knowledge of job hazards, proper maintenance procedures, first aid procedures, and cleanup methods, and that they know how to correctly use respiratory protective equipment and eye protective equipment.

(c) Information as required shall be recorded on a "Material Safety Data Sheet" as specified in Appendix IV or on a similar form approved by the Occupational Safety and Health Administration, US Department of Labor.

Section 6 - Work Practices

(a) Engineering Controls

(1) The employer shall use engineering controls wherever feasible to prevent or minimize exposure of employees to oxides of nitrogen in the workplace.

(2) A closed process for handling the oxides of nitrogen is the preferred engineering control and shall be used wherever possible to prevent contamination of the work area.

(3) An enclosed process with exhaust ventilation is the next preferred means of engineering control of oxides of nitrogen and shall be used wherever possible if a closed process is not feasible. These systems shall be designed and constructed to prevent the escape of oxides of nitrogen into workplaces. Also, appropriate measures are to be taken to ensure that the discharge of oxides of nitrogen to the outdoor atmosphere is in accordance with applicable federal, state, and local environmental pollution control laws and regulations.

(4) General (dilution) ventilation of the worksite is the least desirable means of engineering control of oxides of nitrogen. It shall be used wherever enclosed processes with exhaust ventilation are not feasible. General (dilution) ventilation systems shall be designed and constructed to ensure that exhaust air containing oxides of nitrogen is not recirculated into the work area.

(b) Enclosed Spaces

(1) Persons who must enter enclosed spaces not known to be safe, such as tanks, reaction vessels, and small rooms which contain oxides of nitrogen or wherein oxides of nitrogen may be liberated, shall wear either a combination type C supplied-air respirator operated in the continous-flow mode (positive pressure) or pressure-demand mode (positive pressure) and auxiliary self-contained breathing air supply operated in the pressure-demand mode (positive pressure) equipped with a full facepiece, or a self-contained breathing apparatus operated in the pressure-demand mode

(positive pressure) equipped with a full facepiece. At least one standby person, equipped with proper rescue equipment including a self-contained breathing apparatus which operates in the pressure-demand mode (positive pressure) and having a full facepiece, shall be present outside the enclosed space for emergency rescue of persons inside the confined space. Communications (visual, voice, signal line, telephone, radio, or other suitable means) shall be maintained by the standby person with those inside the enclosed space. Persons inside the enclosed space shall be equipped with safety harnesses and safety lines to facilitate their removal.

(c) Agricultural Silos

(1) The silo ventilation blower shall be operated for at least 30 minutes prior to entrance into the silo and continuously while persons are inside the silo.

(2) Persons who must enter a silo in which the concentrations of nitrogen oxides and oxygen are not known to be safe shall be equipped with respirators and safety harnesses as prescribed in paragraph (b) (1) of this Section. At least one standby person as described in paragraph (b) (1) of this Section shall be present outside the silo for emergency rescue of persons inside the silo. Communications as described in paragraph (b) (1) of this Section shall be maintained between persons inside the silo and the standby person.

(d) Emergencies

(1) Written procedures shall be prepared for emergencies, such as a massive release of oxides of nitrogen to the work area, fire, or explosion.

(2) The employer shall ensure that employees are familiar with procedures covering emergencies.

(3) Self-contained breathing apparatus operated in the pressure-demand mode (positive pressure) and equipped with a full facepiece shall be stored so that they are immediately available to persons who need them to escape from an area where an emergency may occur.

(4) Persons who must enter an area where an emergency has occurred to carry out cleanup operations, maintenance, or repair, shall be equipped with respirators and safety harnesses as prescribed in paragraph (b) (1) of this Section. Also, at least one standby person as described in paragraph (b) (1) of this Section shall be present in a safe place near the area where the emergency has occurred for possible rescue of persons inside the hazardous area. Communications as described in paragraph (b) (1) of this Section shall be maintained between persons inside the hazardous area and the standby person.

(5) Only a self-contained breathing apparatus operated in the pressure-demand mode (positive pressure) and equipped with a full facepiece shall be used by a person engaged in firefighting.

(6) Eyewash fountains and drench-type safety showers shall be readily available in areas where liquid forms of the oxides of nitrogen are being handled. This equipment shall be inspected and tested at least every 30 days to insure proper operation.

(e) Preventive Maintenance

(1) The employer shall carry out a preventive maintenance program which shall include regular and periodic inspection of equipment for leakage of oxides of nitrogen into work areas and immediate repair of leaking equipment.

(2) The employer shall ensure that containers of oxides of nitrogen are stored properly to minimize the escape of oxides of nitrogen into work areas.

(3) The employer shall ensure that worksites contaminated by liquid oxides of nitrogen are cleaned up promptly.

(f) Sanitation

General plant housekeeping should be of high standards with emphasis on cleanup, inspection, repair of equipment and leaks, and proper storage of materials. Escape routes and oxides of nitrogen control equipment shall be kept clear. Sanitation practices shall meet the requirements of 29 CFR 1910.141.

Section 7 - Monitoring and Reporting Requirements

(a) No Occupational Exposure to Oxides of Nitrogen

(1) Workers employed in areas where oxides of nitrogen are manufactured, stored, transported, handled, or produced as byproducts of work operations shall not be considered as occupationally exposed to oxides of nitrogen if it has been determined, on the basis of an industrial hygiene survey carried out by the employer or the judgment of a compliance officer, that workers are not exposed to oxides of nitrogen greater than half of the 1-ppm ceiling limit of nitrogen dioxide or half of the 25-ppm TWA limit of nitric oxide.

(2) The employer shall keep records of any industrial hygiene survey including justification of the conclusion that occupational exposure to oxides of nitrogen are not greater than or equal to half of the 1-ppm ceiling limit of nitrogen dioxide or half of the 25-ppm TWA limit of

nitric oxide. The records shall be retained for at least 20 years after termination of an individual worker's employment.

(3) An industrial hygiene survey of the work area shall be made when equipment changes, process modifications, or worksite changes may reasonably be expected to increase the concentrations of the oxides of nitrogen above the levels defining occupational exposure.

(b) Occupational Exposure to Oxides of Nitrogen

(1) If, from the results of an industrial hygiene survey or the judgment of a compliance officer, there is "occupational exposure" to oxides of nitrogen, the employer shall monitor the environmental concentrations of the oxides of nitrogen by the procedures described in Appendix I at least semianually, except as indicated by a professional industrial hygienist, with procedures described in Appendix I.

(2) The first environmental monitoring of the worksite shall be completed within 6 months of the promulgation of a standard incorporating these recommendations.

(3) Environmental monitoring of the workplace shall be carried out by the employer within 30 days after installation of a new process or within 30 days after equipment changes, process modifications, or worksite changes.

(4) If the measurements of the environmental concentrations of oxides of nitrogen in a work area indicate that the levels of either nitrogen dioxide or nitric oxide exceed the prescribed limits listed in paragraphs (a) and (b) of Section 1, the employer shall install appropriate engineering controls and shall monitor the environmental concentrations of oxides of nitrogen in the work area at intervals of 15

days until the results of at least 2 consecutive monitorings have demonstrated that the environmental concentrations of oxides of nitrogen are below the prescribed limits.

(5) The minimum number of representative ceiling determinations for nitrogen dioxide and TWA exposure determinations for nitric oxide for an operation or process shall be based on variations in exposures and production schedules, considering the number of workers exposed as suggested in Table I-2, and as indicated by a professional industrial hygienist.

(6) The employer shall keep records of all environmental monitoring of oxides of nitrogen. The records shall include not only the determined concentrations of oxides of nitrogen but also shall include a description of monitoring and analytical methods. In addition, the records shall include a listing of the type of respirator and other personal protective equipment, if any, worn by employees. The monitoring records shall identify the employees for whom breathing zone air samples were collected, and the employer shall make such records available to representatives of the Secretary of Health, Education, and Welfare, of the Secretary of Labor, and to the employee or former employee.

(7) The employer shall keep the records of all environmental monitoring of the levels of oxides of nitrogen for each employee for whom breathing zone air samples were collected for at least 20 years after the employee's employement is terminated.

TABLE I-2

SAMPLING SCHEDULE

Number of Employees Exposed to Oxides of Nitrogen	Minimum Number of Air Samples to be Collected and Analyzed
1 - 20	50% of the total number of workers
21 - 100	10 plus 25% of the excess over 30 workers
over 100	30 plus 5% of the excess over 100 workers

II. INTRODUCTION

This report presents the criteria and the recommended standards based thereon which were prepared to meet the need for preventing occupational diseases arising from exposure to oxides of nitrogen (nitrogen dioxide and nitric oxide). The criteria document fulfills the responsibility of the Secretary of Health, Education, and Welfare, under Section 20 (a) (3) of the Occupational Safety and Health Act of 1970 to "...develop criteria dealing with toxic materials and harmful physical agents and substances which will describe... exposure levels at which no employee will suffer impaired health or functional capacities or diminished life expectancy as a result of his work experience."

The National Institute for Occupational Safety and Health (NIOSH), after a review of data and consultation with others, formalized a system for the development of criteria upon which standards can be established to protect the health of workers from exposure to hazardous chemical and physical agents. It should be pointed out that any criteria and recommended standard should enable management and labor to develop better engineering controls resulting in more healthful work practices and should not be used as a final goal.

These criteria for standards for oxides of nitrogen are in a continuing series of criteria developed by NIOSH. The proposed standards apply only to the processing, manufacture, and use of the oxides of nitrogen, or to their release as intermediates, byproducts, or impurities therefrom as applicable under the Occupational Safety and Health Act of 1970.

Oxides of nitrogen are important and fairly common hazards of the workplace and are also among the most important and frequently encountered contaminants in the general atmosphere. Produced by processes involving high temperatures, they are important constituents of vehicular exhaust gases, electric power generating plant emissions, agricultural silos, and other operations involving combustion. Recognition of the potential sources of nitrogen oxides is important and is a prerequisite to the design of measures for control of occupational exposures.

The development of the recommended standard for occupational exposure to oxides of nitrogen has revealed deficiencies in data in the following areas:

(1) Carefully controlled cross-sectional epidemiologic studies concerned with chronic pulmonary changes and hematologic changes as well as studies on the incidence of chronic obstructive pulmonary diseases in workers exposed to low concentrations of oxides of nitrogen.

(2) Chronic effects in experimental animals exposed to oxides of nitrogen according to schedules and concentrations of exposure which simulate the occupational environment.

(3) The role of oxides of nitrogen in the production of teratogenic, mutagenic, and carcinogenic changes in animals.

(4) The possible production of neoplasms from exposure to oxides of nitrogen in combination with hydrocarbons, fibrous dusts, and organic solvents at concentrations observed in the occupational environment.

III. BIOLOGIC EFFECTS OF EXPOSURE

Extent of Exposure

(a) Properties of Oxides of Nitrogen

Since nitrogen dioxide is frequently produced from the oxidation of nitric oxide, some consideration of the relationships of these gases is indicated. Selected chemical and physical properties of nitric oxide and nitrogen dioxide are given in Table XIII-1. [1]

Nitrogen dioxide (NO2) is one of several oxides of nitrogen. It is a reddish brown or dark orange gas with a formula weight of 46.01. Its dimer, nitrogen tetroxide (N204), is colorless. At temperatures between -9.3 C and 135 C, nitrogen dioxide and nitrogen tetroxide coexist as a mixture of gases. Below -9.3 C, a colorless solid consisting of nitrogen tetroxide is formed, while above 135 C, the gas consists mostly of nitrogen dioxide. In evaluations of occupational exposures to the mixtures of these two compounds, however, the results are customarily expressed in terms of nitrogen dioxide.

The conversion of molecular nitrogen into nitrogenous compounds is known as fixation of nitrogen. In the upper atmosphere this is brought about through photochemical processes in which nitrogen and oxygen atoms from dissociated molecules combine to form nitric oxide. At ground level, the combination of nitrogen and oxygen takes place thermally in flames, explosions, and in electric discharges. According to Jacobs, [2] the formation of nitric oxide by these reactions is given by the following equation:

$$N2 + O2 + heat = 2NO$$

Ultraviolet energy or sufficiently high temperatures to accomplish this in the occupational environment are encountered in electric or gas welding, in the combustion of fuels, eg, in furnaces or internal combustion engines, and in the detonation of explosives. [3,4,5,6,7] Nitric oxide is also produced when nitrogen dioxide is dissolved in warm water. The net reaction is:

$$3NO2 + H2O = 2HNO3 + NO \quad [2]$$

Nitrogen dioxide is formed by the spontaneous oxidation of nitric oxide in air at ordinary temperatures, the equation for this reaction is given by:

$$2NO + O2 = 2NO2 \quad [2]$$

Both nitric oxide and nitrogen dioxide are formed when nitric acid reacts with reducing agents. For dilute nitric acid, the resultant mixture contains predominantly NO, while for concentrated nitric acid more NO2 is produced. [2]

Nitric oxide and nitrogen dioxide rarely exist independently in the occupational environment. The rate at which nitric oxide in air is oxidized to nitrogen dioxide is expressed as:

$$d(NO2)/dt = K(O2)(NO)^2$$

where (NO2) and (O2) represent the concentrations of nitrogen dioxide and oxygen, $(NO)^2$ represents the square of the nitric oxide concentration, t is time, and K is a constant for any temperature ($K = 14.8 \times 10^9$ at 20C). [8,9]

Since the rate of oxidation is dependent upon the square of the nitric oxide concentration, oxidation is much more rapid at high concen-

trations. The significance of this was demonstrated by Elkins [9] who, in 1946, calculated that with a nitric oxide concentration of 200 ppm, nitrogen dioxide would be formed at a rate of about 11 ppm/minute. If the nitric oxide concentration were 100 ppm, the oxidation rate would drop to 2.8 ppm/minute, while with 25 ppm of nitric oxide, it would take over 5 minutes for 1 ppm of nitrogen dioxide to be formed. For this reason, both nitric oxide and nitrogen dioxide should be sampled simultaneously. Additional data on the theoretical oxidation rate of nitric oxide in air are presented in Table XIII-2.

The presence of other factors, such as moisture, metal fumes, or ultraviolet radiation, may either accelerate or slow down the actual rate of oxidation of nitric oxide in air. [10] As a result, it is not practicable to estimate the concentration of one of the nitrogen oxides solely on the basis of a measurement of the other.

Mixtures of the oxides of nitrogen are also produced in other ways, such as from the combustion of nitrogen-containing materials or from reactions of nitric acid with metals or organic matter. At the time of release, these mixtures may contain substantial percentages of nitrogen dioxide. Wade et al [5] used various sampling techniques and analyses to estimate the mixtures of nitrogen oxides produced by a number of work operations. The results of these analyses are listed in Table III-1. It should be noted that in operations where fixation of nitrogen from the air occurred at high temperatures, the initial product was largely nitric oxide. Somewhat more nitrogen dioxide is to be expected where nitrogen-containing compounds are decomposed. Where nitric acid is a reactant in acid dipping, nitrogen dioxide will be the predominant oxide released.

TABLE III-1

APPROXIMATE DISTRIBUTION OF NITROGEN OXIDES
GENERATED FROM VARIOUS OPERATIONS

Source	NO_2 %	NO %
Carbon arc	9	91
Oxyacetylene torch	8	92
Cellulose nitrate combustion	19	81
Diesel exhaust	35	65
Dynamite blast	52	48
Acid dipping	78	22

From Wade et al [5]

(b) Sources of Exposure

Although nitric oxide is the oxide of nitrogen initially produced in the welding arc or flame, [4,5] its concentration in such operations is only rarely reported. In many field studies [11,12,13] and controlled laboratory experiments, [12,14,15,16] concentrations of nitric oxide have been reported as "nitrous gases" or "nitrogen oxides", whereas other field investigations [17,18] and controlled studies [19,20,21,22] have expressed concentrations of nitric oxide as "nitrogen dioxide." Differences in describing environmental levels, coupled with the variety of sampling and analytical methods used to assess the environments, virtually preclude direct comparison and interpretation between these studies.

In a simulation of cutting torch operations causing toxicity, Norwood et al [23] reported separate figures for concentrations of nitric oxide and nitrogen dioxide in 2 of 10 samples. This study showed nitrogen dioxide to be 13% of the total oxides found 15 minutes after cutting began. The

nitrogen dioxide increased to 33% after an additional 25 minutes. Detailed results are given in Table XIII-3. The measured rate of oxidation to nitrogen dioxide appears to be consistent with the theoretical oxidation rate of nitric oxide in air presented in Table XIII-2.

Theoretical predictions based on the kinetic rate of oxidation of nitric oxide to nitrogen dioxide would not be expected to hold in the case of electric arc welding because of the presence of such additional factors as ultraviolet radiation, ozone, iron fume, and moisture. For example, nitrogen dioxide is dissociated by ultraviolet radiation producing nitric oxide plus oxygen atoms. The oxygen atoms, in turn, react with nitrogen dioxide at a very rapid rate producing nitric oxide and oxygen molecules. [24] Thus, according to Silverman and Husain, [10] ultraviolet rays emitted in arc welding would counteract the oxidation of nitric oxide to nitrogen dioxide. On the other hand, ozone produced in the arc could oxidize nitric oxide to nitrogen dioxide or to even higher oxides, such as nitrogen pentoxide. They also pointed out that iron and moisture present could have a converse influence, reducing nitrogen dioxide to nitric oxide, and possibly to ammonia or nitrogen. In addition, freshly formed iron fumes may react with nitrogen oxides to produce particulate nitrites and nitrates, with a consequent reduction in the amount of gaseous oxides present. [10]

In their own welding experiments, conducted in a test chamber, Silverman and Husain [10] found that approximately equal concentrations of nitric oxide and nitrogen dioxide totalling approximately 30 ppm showed no appreciable changes in the ratio of the gases after a period of two to three hours. Here again, at such concentrations, the figures in Table

XIII-2 indicate that very little measurable change would have been expected.

In general, it has been found that hot metals decompose nitric oxide forming the metal oxide and nitrogen. [10] This may explain the higher rate of formation of oxides of nitrogen when an oxyacetylene flame does not touch metal. [19,23] As shown in Table XIII-4, Norwood et al [23] found that as much as 250 ppm of nitrogen oxides were formed where the flame was not in contact with metal, and only 47 and 71 ppm in tests where stainless steel was being melted. Similarly, Steel and Sanderson [19] reported that the concentrations of nitrogen dioxide produced by flame alone were several times greater than the concentrations observed when metals were being flame-cut (see Table XIII-5).

The changeover from coke oven gas to natural gas in The Netherlands was followed by complaints of toxic effects in glassblowing shops. Van Mourik [25] studied the formation of nitrogen oxides in a glassblowing burner using various combinations of air and oxygen with natural gas, coke, oven gas, methane, and hydrogen. The change to natural gas caused a change in flame characteristics which led to an increased use of oxygen with a consequent increase in flame temperature. The rate of nitric oxide production is rapidly increased if the amount of oxygen in the mixture is increased, [23] as is illustrated in Table XIII-3. Furthermore, van Mourik [25] has pointed out that regardless of the nitrogen content of the gas, approximately the same amount of nitric oxide is formed, provided the flame temperature is constant. Another example [26] of occupational hazard concerns exposure to exhaust gases from ice resurfacing machines in ice arenas. Such exposures made the machine operators, as well as arena

patrons, ill on a number of occasions. Investigations of 45 ice arenas in Minnesota showed nitrogen oxides concentrations (reported as nitrogen dioxide) as high as 40 ppm. However, concentrations of carbon monoxide were elevated, suggesting that nitrogen oxides were not the only cause of adverse effects. Since the form of nitrogen emitted from internal combustion engines exists largely as nitric oxide at the time of discharge, workers employed in automobile parking or repair garages may have occupational exposures.

The production of nitric oxide by the catalytic oxidation of ammonia is the first step in the manufacture of nitric acid. [27] Information on occupational exposures to nitric oxide from such operations has not been found. Similarly, no published information on nitric oxide exposures in other industrial operations has been found, other than those presented in Tables III-1, XIII-3, and XIII-4.

As reported by Kennedy, [28] underground blasting operations with both nitro-explosives and gunpowder produce levels of the oxides of nitrogen up to 88 ppm after normal firing of a commonly used blasting explosive, and up to 167 ppm after "misfires" (incomplete detonation). Conventional powder shots may cause momentary concentrations of up to 56 ppm and concentrations as high as 150 ppm after multiple firings.

Commins et al [29] noted concentrations of nitric oxide and nitrogen dioxide reaching several hundred ppm were produced in a freshly filled agricultural silo during various stages of the decomposition process. The maximum concentrations of nitric oxide and nitrogen dioxide were found on the fifth day after loading the silo. At that time, a distinct layer of brown gas was observed at the center of the silo. In general, con-

centrations decreased with increased height above the surface of the silage. Data are presented in Table XIII-6. Measurements were also made before and after operation of the silo filling blower. Use of the blower did reduce the levels of nitric oxide and nitrogen dioxide; however, high concentrations of these gases still remained near the surface of the silage.

It is difficult to estimate how many people are exposed to oxides of nitrogen since hazardous occupations also involve all workers located in the vicinity of operations or processes having conditions conducive to the generations of nitrogen oxidation, ie, furnaces, boilers, welding, internal combustion engines, and nitrogen oxides produced from numerous chemical processes. The difficulty in delineating the incidence of exposure has been shown by Storlazzi [30] in a study of welding and burning operations in US naval shipyards. Although only 6,000 workers were directly engaged in such activities, approximately 60,000 workers were indirectly exposed to the byproducts of these operations. NIOSH estimates that 1,500,000 workers are potentially exposed to the oxides of nitrogen. In view of the factors mentioned, this figure is low.

An estimated [3] 200,000 tons of nitrogen oxides are produced annually from industrial processses and ten million tons result from fuel combustion. These are rough estimates at best and do not distinguish between the various nitrogen oxides. From the many cases found in the medical literature, examples of which are given under Historical Reports, it is known that serious, even lethal, concentrations of nitrogen oxides may be encountered. Similar serious consequences have been reported from

chemical processes. [31-36]

Historical Reports

One of the earliest accounts of what must have been primarily exposure to nitrogen dioxide is that of Desgranges [31] in 1804. Two carboys of concentrated nitric acid broke in a storeroom, reacted with a quantity of wood, and produced a sensation described as suffocating. The merchant entered the room for two periods of approximately five minutes each in close succession. He experienced an immediate sensation of suffocation and his hair turned yellowish red. He recovered but four hours later he became increasingly dyspneic and had painful sensation of constriction in the epigastric region. Thirteen hours after exposure, he appeared much better, but three hours later he suddenly became cyanotic and later delirious. Approximately 27 hours after exposure, he died in severe respiratory distress.

There were several subsequent reports of persons acutely exposed to the vapor of nitric acid reacting with wood or other organic material, eg, Zadek, 1916, [32] or with metals, eg, Fraenkel, 1902, [37] Wood, 1912, [38] Hortsch, 1942, [33] Darke and Warrack, 1958, [34] in many cases with fatal results. In all these cases, the onset of serious illness was characteristically delayed for several hours, after which severe pulmonary edema developed.

In 1913, Lehmann and Hasegawa [39] reported on toxic effects in cats and rabbits exposed to nitrous gases (probably nitric oxide, nitrogen dioxide, and nitric acid fumes) at concentrations between 41 and 2,039 ppm. The nitrous gases were produced by one of two methods. In the first

method, gases produced by the reaction of nitric acid and copper were collected in a gasometer over water, transferred in a tenfold dilution with hydrogen into a paraffin oil gasometer, and then mixed with a stream of fresh air. In the second method, a small amount of air was aspirated by smoking nitric acid and then mixed with a current of fresh air. Airborne concentrations, expressed as concentrations of nitrous acid, were determined by oxidation of samples by hydrogen peroxide or by chemical reduction using potassium iodide. Cats exposed at concentrations of 41, 64, and 57 ppm for 3, 6, and 7 hours, respectively, did not show any signs during exposure or macroscopic lung changes after the animals were killed. Cats exposed at 117 ppm and above for periods from approximately 1 to 8 hours showed definite changes in respiration, and most animals died during exposure. At necropsy, pulmonary edema and methemoglobinemia were observed. Rabbits exposed at identical concentrations did not show the same signs as the cats.

In this study, [39] Hasegawa subjected himself on three separate occasions to nitrous gases at average concentrations between 62 and 158 ppm. After a 1-hour exposure at a concentration of 62 ppm, he noted laryngeal irritation and an increase in respiration rate. There were no ill effects following this exposure. In another experiment, the concentration was increased to 158 ppm. Hasegawa reported that he had to leave the room after 10 minutes of exposure. His symptoms included a feeling of suffocation, considerable coughing, mucous secretion in the nose, and tearing, all of which subsided 7 hours after exposure.

Later, accounts began to appear of severe exposures to "nitrous fumes" arising from the explosion [35] or the combustion [36] of nitro-

explosives, resulting in severe, sometimes fatal, pulmonary edema.

Severe "nitrous fume poisoning" was reported in arc welders by Adler-Herzmark [40] in 1929, and later in oxyacetylene-torch cutters by Norwood et al. [23]

The additional entity of bronchiolitis fibrosa obliterans, seen by Fraenkel [37] in 1902 in a brassfounder exposed to fumes from the pickling of castings in a mixture of nitric and sulfuric acids, has continued to be reported as a late sequel of poisoning by nitrogen oxides often following recovery in acute pulmonary edema from one [34] to three [35] or even six [41] weeks.

Peterson et al [42] in 1949 reported that toxic agents generated under certain circumstances within agricultural silos contained a high proportion of nitrogen dioxide. Exposures to these agents have resulted in "silage gas poisoning" [43] or "silo-filler's disease", [44] the symptoms of which were characteristic of nitrogen oxides poisoning including, in some cases, the sequelae bronchiolitis fibrosa obliterans. [44]

Effects on Humans

Tables XIII-7 and XIII-8 summarize clinical, epidemiologic, and experimental effects noted in humans exposed to nitrogen dioxide and nitric oxide. Evaluative or qualifying information on each study is included under the section entitled remarks.

In 1916, Zadek [32] described a group of cases of mixed nitrogen oxides poisoning with clinical and pathologic findings which were interpreted by von Oettingen [45] as effects of nitric oxide. Several carboys of crude nitric acid exploded following contact with burning wood

shavings and excelsior in a factory fire. Of the 20 firemen exposed to what was described as dense greenish fumes, 11 were hospitalized the following day with severe headache, gastrointestinal and respiratory symptoms which appeared as early as 3 1/2 hours after exposure. Of the three most severe cases, one had nitrous acid detectable in his urine, two had detectable nitrite in the sputum, and all had spectroscopically demonstrable methemoglobinemia. One of these men died in coma about 2 days after exposure. An autopsy revealed severe lung injury in addition to methemoglobinemia. The other two severe cases, with both clinical and radiologic evidence of pneumonitis, recovered after 4-6 days. The remaining 17 men who were exposed made a fairly rapid recovery from their milder respiratory and gastrointestinal symptoms.

In 1912, Wood [38] described a case of fatal pneumonia of delayed onset following the inhalation of gas from nitric acid acting upon cadmium-silver alloy. This paper also reviewed 26 other cases from the foreign literature. However, as is often the case in the early literature, "nitric oxide" was clearly used as a nonspecific term. The author's own case, as described above, corresponds closely to the now well-known features of mixed oxides of nitrogen poisoning.

In 1930, Flury [46] presented a theoretical review of the literature on poisoning by "nitrous gases" (defined as nitric oxide, nitrogen dioxide, nitrogen tetroxide, nitrous and nitric acids, in varying proportions). This paper laid the foundation for distinguishing toxicity of nitric oxide from the higher oxides of nitrogen. Flury observed that both human and animal poisoning by "nitrous gases" fell into several distinct clinical categories including: (1) irritant-gas type, (2) reversible type, (3)

shock type, and (4) combined type. The irritant-gas type was characterized by an initial local irritation which, after a symptom-free latent period of several hours, progressed to breathlessness and cyanosis. Death occurred from pulmonary edema after 1-2 days. Immediate onset of pulmonary edema and death within a few minutes to a few hours after exposure was observed less frequently. Cases in which the illness was reversible showed breathlessness, cyanosis, vomiting, giddiness, stupefaction, delirium, fainting, unconsciousness, and severe methemoglobinemia. If the subject was promptly removed from the exposure environment, pulmonary edema did not ensue and recovery was complete. The shock type of illness showed almost instantaneous asphyxia, convulsions, and respiratory arrest. According to Flury, [46] death resulted from interruption of the pulmonary circulation. The combined type displayed symptoms characteristic of both types 1 and 2. Flury described experiments with white mice in which the distinctive effects of nitric oxide and nitrogen dioxide could be demonstrated individually. These experiments were repeated and elaborated on by Pflesser [47,48] some years later and are described under Animal Toxicity.

Nitrogen dioxide gas is an irritant to the mucous membranes and its inhalation may cause coughing, sometimes severe, which may be accompanied by mild or transient headache. [41,49] Mild dyspnea may also be present during exposure. [23] After less severe exposure, the symptoms of irritation usually subside completely for several hours. [34,49] In some cases, symptoms may persist in a mild form for several hours [23] or even days, [41] after which recovery may occur but, more commonly, if exposure to nitrogen dioxide has been severe enough, acute pulmonary edema ensues.

Signs and symptoms include dyspnea, cough, cyanosis, and upon auscultation, moist rales are heard at the base of the lungs. [23,49] Acute pulmonary edema is usually preceded by an interval of several hours during which few, if any, symptoms appear. Many fatalities occur because of the suddenness and severity of effects and the characteristic delay in onset of up to 12 hours, by which time the exposed subject may be at home and remote from prompt medical attention. [32,33,36,40]

In some cases, severe and increasing dyspnea with fever and cyanosis ensues, usually occurring after an interval of several days to 6 weeks following recovery from the initial, though delayed, acute pulmonary edema. [34,41,44,50,51] This condition has, on occasion, developed long after the exposure, without clinical evidence of pulmonary edema. [35,52,53] This has been called bronchiolitis fibrosa obliterans on the basis of microscopic findings at necropsy. [34,53] These effects have been reported following exposure to mixed oxides of nitrogen generated from: (1) the reaction of nitric acid with various metals, [34,37,49] (2) the explosion of a vessel containing red fuming nitric acid, [53] (3) an oxyacetylene burner, [52] (4) "nitrogen dioxide gas leaking in a chemical plant," [41] and (5) gases generated under certain circumstances in agricultural silos. [44,50,51] Silo gases have been reported to contain nitrogen dioxide (35-1920 ppm), nitric oxide (30-630 ppm), and carbon dioxide (25-60% v/v). [29]

Whether or not sequelae of chronic bronchiolitis fibrosa obliterans are diagnosed or found depends upon the severity of the lesion, the sensitivity of the means of detecting residual effects, and upon individual variations. Tse and Bockman [41] in 1970 reported four cases of bronchiolitis in firemen who were exposed to nitrogen dioxide originating

from a leak in a chemical plant. Three of the men recovered completely after six to seven weeks, ie, they had no symptoms, clinical signs, or pulmonary dysfunction, except that one had a moderately decreased diffusing capacity at six weeks after exposure. The fourth man continued to complain of dyspnea following exertion, 18 months after exposure. Serial lung function studies on this man over an 18-month period showed a progressive decrease in vital capacity with an increase in residual volume. Both the maximal breathing capacity and the lung compliance were decreased. In addition, studies revealed a decrease in arterial oxygen partial pressure. These results indicated the presence of uneven ventilation with both obstructive and restrictive impairments.

In 1957, Becklake et al [54] reported on followup studies of seven selected patients who had recovered from an episode of acute pulmonary edema following exposure to oxides of nitrogen in various mine-blasting accidents. The study periods were up to 64 months after the exposures. One patient complained of mild dyspnea, and four complained of definite breathlessness on exertion. Five cases showed a decrease in maximal breathing capacity and an increase in the "non-elastic work of breathing." The other two subjects claimed to have recovered completely from the accident. One of these two had completely normal lung function, while the other had high nonelastic resistance but normal maximal breathing capacity. It was suggested [54] that the six subjects who demonstrated residual abnormalities had some degree of bronchial and bronchiolar narrowing due to the fibrotic changes of bronchiolitis obliterans.

In 1971, Ramirez and Dowell [50] reported a followup of a case of "silo-filler's disease." Seven years after the incident, the subject's

chest X-ray showed diffuse reticular and fine nodular markings. Except for mild hypoventilation and hypoxemia, his pulmonary function remained normal. Lung compliance and airway resistance remained normal throughout the course of followup and no impairment of maximal voluntary ventilation or expiratory flow rates were noted.

In 1973, Scott and Hunt [51] reported on four episodes of "silo filler's disease" in three farmers who were observed at 10, 28, 75, and 327 days following exposure. Serial pulmonary function tests showed acute obstructive, restrictive, and diffusion defects which cleared almost completely within the observation periods. Summarizing their review of cases of nitrogen oxides poisoning sequelae, the authors [51] concluded that chronic pulmonary insufficiency occurs following silo gas exposures in patients with preexisting "small airway disease" (predominantly chronic bronchitis and emphysema). They further stated that "animal exposure studies and lung biopsies in humans lead to speculation that the chronic disease produced might be centrilobular emphysema."

Whether or not exposure to nitrogen dioxide causes methemoglobinemia in man is still a matter of controversy. A comprehensive review article on the chemistry and pharmacology of methemoglobinemia was published by Bodansky [55] in 1951. In most cases in which methemoglobinemia was inferred or reported directly, [32,56,57] the exposures have been to mixed oxides of nitrogen with circumstantial evidence that nitric oxide was present.

The only direct information found on the effect of nitric oxide in man is furnished by a report on a contamination of anesthetic nitrous oxide by nitric oxide. The extent of contamination was greater than 1.5%. [58]

The contaminated gas was administered to 2 female surgical patients in the course of routine general anesthesia. The first patient received a 75% concentration of the nitrous oxide in oxygen. After about 3 minutes of inhalation, cyanosis developed and rapidly became more severe, despite an increase of the oxygen to 50%. After a further 20 minutes, the nitrous oxide was discontinued. Electrocardiography showed depression of the ST segment in all leads, a sign of myocardial hypoxia. Chest X-ray screening showed several ill-defined opacities in the lung fields. Blood was obtained by femoral artery puncture and was brown in color, suggestive of methemoglobinemia. Later the presence of methemoglobin in the blood was confirmed in the laboratory. Methylene blue (10 ml of a 1% solution) was given intravenously and methemoglobin was not detected in 2 subsequent blood samples, taken 4 1/2 and 8 hours later. The patient died of cardiac arrest approximately 18 1/2 hours after the commencement of exposure to the nitric oxide. At autopsy, severe pulmonary edema was confirmed.

Before the contamination of the nitrous oxide was realized a second patient was induced with the same gas mixture. The patient became cyanotic within a short time. After only a few minutes the administration of nitrous oxide was discontinued and 100% oxygen was administered from a different cylinder. She showed some signs of respiratory distress but she later recovered fully.

Commins et al [29] in 1971 reported that the toxic gas mixture periodically present above fermenting silage and responsible for "silo fillers disease" may contain a high proportion of nitric oxide, in addition to nitrogen dioxide and carbon dioxide (see Table XIII-6). The findings described, including respiratory irritation, delayed pulmonary edema, and

bronchiolitis fibrosa obliterans were, in the opinion of many authors, [43,44,50,51,59,60,61] largely attributable to the action of the high concentration of nitrogen dioxide.

An experimental study on human subjects on the retention of nitrogen dioxide was reported in 1970 by Wagner. [62] Seven subjects were exposed to nitric oxide at several different concentrations (5, 1, 0.5 and 0.33 ppm) under conditions of normal and of maximal oral respiration. By comparative analyses of inhaled and exhaled gases, the percentage absorption of the nitric oxide was measured. The retention was uniformly high (from 85 to 93%), seemingly independent of concentration within the range tested, and somewhat higher with maximal as opposed to normal respiration. A parallel series of studies was conducted with nitrogen dioxide. Absorption rates were similar to those reported for nitric oxide.

Despite the fact that nitrogen dioxide is an irritant gas, there are few primary references in the literature as to its effects upon the eyes or mucosae other than that on the lower respiratory tract. In 1970, Morley and Silk [63] reported conjunctivitis and pharyngitis occurring in five men welding zinc-plated steel in a confined space. These effects had subsided 18 hours later when the men were reexamined. Representative atmospheric measurements showed total oxides of nitrogen, expressed as nitrogen dioxide, in the 4-20 ppm range with an average of 7.4 ppm (19 measurements). Rodin and Boyenko [64] in 1970 reported a prevalence of 64% of "chronic catarrhal processes in the upper respiratory tract" in 334 arc welders who had up to 10 years of work history. In the workers who had more than 10 years service in welding, 22% had subatrophic rhino-pharyngitis.

Hortsch [33] reported a case of right hemiplegia in a 62 year-old worker following exposure to oxides of nitrogen evolved from pickling metal objects in a 50-50 mixture of nitric and sulfuric acids. The victim also developed pneumonitis within 24 hours and died approximately 3 1/2 days after exposure. Autopsy revealed respiratory lesions and widespread hemorrhages in the brain. It is difficult to attribute the cerebrovascular damage directly to the effects of nitrogen dioxide exposure since the patient had severe hypoxemia, secondary to the pneumonitis, at the time.

An experimental self-exposure was reported by Lehmann and Hasegawa [39] in 1913. Hasegawa inhaled 62 ppm (calculated as nitric acid) for 1 hour and reported only slight irritation of the larynx and an objectionable odor, but no other overt ill effects. He inhaled 75-100 ppm for 1 hour, followed immediately by 25-75 ppm for another hour, and reported irritation with cough and an increase in pulse and respiratory rates. He was able to tolerate a concentration of 158 ppm for only 10 minutes because of coughing, irritation of the nose and throat, lacrimation, headache, nausea, and vomiting. However, he rested well after this exposure and there were no delayed aftereffects noted.

The human odor threshold for nitrogen dioxide was investigated by Henschler et al. [65] Those described as olfactorily sensitive could smell as little as 0.1 ppm of nitrogen dioxide, more than half the subjects could detect 0.2 ppm, and all recognized 0.4 ppm. However, even at 4.0 ppm not all subjects recognized the gas as nitrogen dioxide. Olfactory fatigue was observed to develop very rapidly, so that the gas was smelled for only 10 minutes at 4.0 ppm and for five minutes at 0.4 ppm. In 1970, Rumsey and Cesta [66] reported that the mean odor threshold for nitrogen dioxide in 10

volunteers was 0.5 ppm or less.

In 1967, Abe [67] reported on experimental exposures of five healthy adult men to nitrogen dioxide at 4-5 ppm for 10 minutes. Concentration of the nitrogen dioxide was measured by simultaneous use of the Saltzman method and Kitagawa-type detection tubes. Measurements of "effective lung compliance," "inspiratory maximum viscous resistance," and "expiratory maximum viscous resistance" were made prior to the gas inhalation, immediately after exposure, and at intervals of 10, 20, and 30 minutes after inhalation had ceased. Values for effective compliance obtained 30 minutes after the cessation of exposure showed a tendency to decrease by 40% as compared with controls. Expiratory and inspiratory maximum viscous resistance were unchanged immediately after completion of exposure but gradually increased from 10 minutes after exposure and reached a maximum at 30 minutes.

In 1971, von Nieding et al [68] described the effects of low concentrations of nitrogen dioxide on the respiratory gas exchange and airway resistance in patients with chronic bronchitis. Eighty-eight chronic bronchitis patients, aged 34-72 years, breathed a nitrogen dioxide-air mixture containing from 0.5 to 5.0 ppm (as measured by the Saltzman method) either for 15 minutes or for a total of 30 breaths. Inhalation of nitrogen dioxide concentrations between 1.5 and 5.0 ppm increased airway resistance significantly. Lower concentrations had no significant effect. While the end-expiratory alveolar oxygen tension remained nearly constant during exposure at 4 and 5 ppm nitrogen dioxide, a significant decrease of the arterial oxygen tension and a corresponding increase of the end-expiratory arterial pressure difference for oxygen occurred. After

inhalation of 2 ppm of nitrogen dioxide, there was no decrease in the arterial oxygen tension.

In 1973, von Nieding et al [69] reported an extension of their earlier studies. In 16 healthy male volunteers, carbon monoxide diffusing capacity was measured by the single-breath method, before and after inhalation of 5 ppm nitrogen dioxide for 15 minutes. A statistically significant ($p < 0.01$) decrease in the diffusing capacity for carbon monoxide by an average of 3.8 ml/0.1 min/0.1 torr (from 20.6 to 16.8) was observed. In 14 patients with chronic bronchitis, the arterial oxygen partial pressure was significantly depressed after 15 minutes of exposure to nitrogen dioxide at a concentration of 5 ppm. A corresponding increase in alveoloarterial oxygen pressure gradients was observed. Continued exposure for 60 minutes indicated no significant disturbances of respiratory gas exchange beyond that noted after 15 minutes of exposure. An increase in relative airway resistance was observed in 70 chronic bronchitic patients after inhalation (30 breaths) of nitrogen dioxide above 15 ppm. Relative airway resistance remained unchanged below this concentration.

Epidemiologic Studies

In 1937, Vigdortschik et al [70] reported a study of "the symptomatology of chronic poisoning with oxides of nitrogen" in 127 printing shop and sulfuric acid plant workers reportedly exposed at levels generally below 2.8 ppm. The clinical signs and symptoms reported and attributed to "oxides of nitrogen" included: dental erosion and gingivitis; emphysema and compensated pulmonary tuberculosis; cardiovascular hypotonia and

bradycardia; polycythemia rubra, granulocytosis, and basophilia; decreased osmotic fragility of red blood cells and accelerated agglutination of the blood cells; and reduced catalase index, reduced alkali reserve, reduced blood sugar and "lability of the blood sugar curve." The presence of dental erosion in many of these workers suggests that the workers were also exposed to mists of sulfuric or nitric acid. Furthermore, the authors did not describe the sampling methods, sampling locations, or analytical methods employed. Because of these deficiencies, the relevance of this study is questionable.

In 1972, Kosmider et al [71] published a study of 70 men, aged 26-48, exposed in a chemical plant for 6-8 hours daily for 4-6 years to what was described as only oxides of nitrogen. The authors reported concentrations of oxides of nitrogen between 0.4 and 2.7 ppm as nitrogen dioxide. There was no information on analytical method, sampling methods, location of sampling, frequency of sampling, the possible presence of other contaminants, or other information allowing inference or judgment as to how, or to what extent, the environment was characterized. A control group was selected consisting of 80 men of similar ages who were not exposed to oxides of nitrogen. All workers smoking more than ten cigarettes daily were excluded from both groups. The men exposed to nitrogen dioxide complained of sporadic cough with mucopurulent expectoration and dyspnea on exertion. Fine bubbling rales and "whistling" sounds were heard in some men, primarily over the lower lungs. There were no chest X-ray abnormalities. Spirometry showed slight, statistically insignificant reductions in vital capacity and maximum respiratory volume. There was an insignificant decrease in the group's mean blood pH. Carbon dioxide

partial pressure and total carbonic acid in the blood were increased. Total serum proteins were significantly below that of the controls. There was a reduction in albumin and gamma-globulin, an increase in the alpha-1-, alpha-2-, and beta-globulins, and a statistically significant increase in the urinary hydroxyproline and acid mucopolysaccharide excretions. The authors [71] concluded from these clinical findings as well as from their animal data (see Animal Toxicity) that long-term exposure at such levels of oxides of nitrogen lead to emphysema in man. The urinary excretions mentioned above were suggested by the authors to be decomposition products of collagen (more likely of collagen and of other elements of connective tissue). They further implied that such tissue destruction is inherent in the pathogenesis of emphysema. Some of the workers were reported to have had chronic bronchitis, but a comparison between control and experimental spirometric changes on a group mean basis was statistically insignificant, a finding which is incompatible with chronic obstructive pulmonary disease. Interesting as this study is, it cannot be given full credence in view of the absence of details on the characterization of the environment, absence of spirometric changes, lack of evidence of a controlled diet in relation to urinary excretion of amino acids and glycoproteins, and omission of supporting evidence on the relation between these urinary excretions and the development of emphysema.

In 1973, Kosmider and Misiewicz [72] reported a rise in the mean total lipid level in the serum of the 70 exposed workers studied in the previous report compared with the 80 control subjects. They found a rise in the mean levels of the beta- and gamma-lipoproteins and a fall in the alpha-lipoproteins and in total serum cholesterol (both free and

esterified) in the exposed workers. The significance of these serum lipid changes in relation to exposure to oxides of nitrogen is not apparent.

In 1972, Kennedy [28] reported a study of the prevalence of emphysema in coal miners exposed sporadically to oxides of nitrogen after underground blasting operations. Some evidence of emphysema was found in 84 of the 100 miners studied. He attributed the emphysema to the nitrogen oxides exposures, but no satisfactory control group was studied to support this conclusion. Comparisons were made with other smaller-scale studies of gold miners who had been exposed only once to "nitrous fumes". The gold miners had a lower incidence of emphysema; however, the use of the gold miners as an adequate control must be questioned. The results of this study are further complicated by the association of coal worker pneumoconiosis with emphysema. [73]

In 1975, French [74] summarized results of a 4-year epidemiologic study concerned with the effects of exposure to nitrogen dioxide in communities located near TNT production plants in Chattanooga, Tennessee. [75,76,77] In 1968-69, the communities were dividied into high (0.083-0.219 ppm), medium (0.063 ppm), and low (0.031 ppm) exposure categories. Over the course of the 4-year study, ambient levels were reduced to 0.031, 0.027, and 0.024 ppm for the high-, medium-, and low-exposure communities, respectively. Results indicated that "lower respiratory" morbidity rates and the incidence of "acute respiratory disease" were significantly higher in the high- and intermediate-exposure communities compared with the low-exposure community, particularly in children below 12 years of age. Significant differences in these findings were also noted between high- and intermediate-exposure communities. There were no significant differences

in the three communities in the prevalence of chronic respiratory symptoms such as chronic bronchitis. It is difficult to attribute the differences in acute respiratory illnesses only to the differences in exposure to nitrogen dioxide since the concentrations of suspended nitrates and total suspended particulates were also increased in the high-exposure as compared with the medium- and low-exposure communities. Suspended sulfates did not differ between communities; however, concentrations of other possible contaminants such as sulfur dioxide were not reported.

In 1955, Vigliani and Zurlo [78] made a brief comment on oxides of nitrogen in a review article on their own research into maximum acceptable concentrations of industrial poisons in the workplace. They stated that workers in 4 catalytic nitric acid plants, exposed for several years to nitrogen dioxide at concentrations averaging from 30 to 35 ppm, had no complaints or signs or symptoms of toxicity. Vigliani and Zurlo expressed the view that the reduction in 1954 by the American Conference of Governmental Industrial Hygienists of their recommended Threshold Limit Value for oxides of nitrogen from 25 ppm to 5 ppm (for nitrogen dioxide alone) was unwarranted. In their opinion, 15 ppm for "nitrous gases," calculated as 50% nitrogen dioxide and 50% nitrogen tetroxide, was acceptable, as long as no ozone was present. Data supporting this conclusion were not included.

Epidemiologic studies of the effects of nitric oxide per se have not been reported. Nitric oxide is probably involved in most occupational exposures to the mixed oxides of nitrogen or "nitrous fumes". Most of these studies [56,57,63] are difficult to evaluate in terms of a dose-response relationship because the environmental data have rarely been

expressed in terms which permit specification of the nitric oxide concentration. In these studies, either the analytical methods did not differentiate between the different nitrogen oxides or the data were expressed entirely in terms of one oxide, [56,57,63] usually nitrogen dioxide. [56,63]

The presence of methemoglobinemia has commonly been held as indicative of nitric oxide poisoning rather than of nitrogen dioxide poisoning. [45,79,80] Such an assumption is suspect in the light of recent animal experiments. [81,82] One epidemiologic study on nitrogen fertilizer plant workers allegedly exposed to carbon monoxide, ammonia, and mixed oxides of nitrogen may be cited. [57] One hundred and seventy workers and 54 controls (mechanical maintenance workers in a woolen mill) were studied on 2 occasions, separated by two years. No environmental data were given but it was implied that the level of oxides of nitrogen (expressed in terms of nitrogen pentoxide) was above the maximum permissible concentration (5 mg/cu m or about 3 ppm in the USSR). [83] The workers had relatively high levels of carboxy- and methemoglobin in their blood as evidence of the effects of these gases. The main finding of this study was that workers under these exposure conditions developed pyridoxine (Vitamin B6) deficiency, but the mechanism for this and the individual roles of the gases were not discussed. [63]

McCord et al [56] found methemoglobin levels in the blood of four arc-welders of 2.3, 2.3, 2.5, and 2.6%, respectively, measured spectrophotometrically. They were exposed to oxides of nitrogen in the 2.0-10.3 ppm range, expressed as nitrogen dioxide. The duration of exposure prior to blood sampling was not given. The normal methemoglobin

level in man is about 1% of total hemoglobin. [84] Even assuming the elevated methemoglobin levels to be due to the nitric oxide exposure of the order of 10 ppm, it seems unlikely that an increase in methemoglobin content of the blood of 1 or 2% would be injurious to health, even on long-term exposure. At what point higher blood levels would pose an occupational hazard is uncertain. Persons who are heterozygous for the trait of hereditary methemoglobin reductase (diaphorase) deficiency have this low order of methemoglobinemia throughout life and are entirely asymptomatic. [84] Moreover, the analytical methods generally available for blood methemoglobin measurements in 1941 were rather inaccurate and a difference of only 1-2% from the physiologic normal would be well within experimental error. [85]

Animal Toxicity

Tables XIII-9 and XIII-10 summarize animal data on the inhalation toxicity of nitric oxide and nitrogen dioxide. It is evident from these tables that exposure to the oxides of nitrogen results in diverse responses across species. An attempt has been made to note these contrasting effects and differences between species in terms of toxic concentrations both in the tables and in the text below.

(a) Nitric Oxide

The first description of animal studies on the toxicity of nitric oxide, as distinct from nitrogen dioxide or mixed oxides of nitrogen ("nitrous fumes"), appears to be that given by Flury [46] in 1930. Describing work performed in Germany during World War I, he outlined an apparatus for the exposure of white mice to a stream of air in which pure

nitric oxide was continuously added. Mice exposed to the gas mixture close to the point of mixing manifested strikingly different toxic effects than those exposed in the same apparatus, but in a more distal section. In the first instance, where the content of unoxidized nitric oxide in the mixture was still relatively high, cyanosis occurred after a few minutes, the red eyegrounds (probably conjunctival or red reflux) became gray-blue, and then breathlessness appeared with paralysis and convulsions. If the mice were promptly removed and exposed to fresh air, they recovered rapidly and completely without apparent sequelae. Mice exposed in the distal part of the apparatus where, according to the author, nitrogen dioxide was predominant showed immediate signs of irritation but no cyanosis and no paralysis. Death resulted from pulmonary edema.

The following year, in their book on toxic gases, Flury and Zernik [86] gave some quantitative data on exposure of mice to nitric oxide, probably from the same experiments. Mice exposed at a concentration of 5,000 ppm died after 6-8 minutes. With inhalation at a concentration of 2,500 ppm, the animals were observed to be on their sides after 6-7 minutes of exposure and died after 12 minutes. Removal of the mice after 4-6 minutes of exposure resulted in full recovery within 24 hours.

These experiments were repeated and described in much fuller technical detail by Pflesser in 1935. [48] The clinical description of the toxic effects and mode of death of white mice exposed predominantly to nitric oxide were identical in every detail to those of Flury [46] and Flury and Zernik. [86] Pflesser [47] noted that at autopsy there was no evidence of lung injury or of pulmonary edema and that spectroscopy of the

blood showed typical methemoglobin lines. He gave the following quantitative data:

> at 3,500 ppm, animals died in 4-5 minutes;
> at 350 ppm, all the animals died;
> at 320 ppm, half the animals died;
> at 310 ppm, all the animals survived an exposure of 8 hours.

By way of contrast, the effects of nitrogen dioxide exposure were quantitatively expressed as follows:

> 1,500 ppm and more was lethal to all animals;
> 1,200 ppm and less was survived by all animals.

These figures indicate a remarkably steep dose-response curve, both for nitric oxide and for nitrogen dioxide. From Pflesser's experiments, it became apparent that nitric oxide was approximately four times more toxic than nitrogen dioxide.

The question of comparative toxicities of these 2 gases is, however, complex. Experiments with albino mice and guinea pigs performed by Paribok and Grokholskaya [87] in 1962 indicated that at concentrations above 1 mg/liter (833 ppm) a 1-hour exposure to nitric oxide was more toxic than the same exposure to nitrogen dioxide. On the other hand, with 8-hour exposures at lower concentrations, nitrogen dioxide produced a higher toxic effect than nitric oxide. For nitric oxide, if the concentration is not high enough to be rapidly lethal, the animal apparently makes a complete recovery. [46,47,86] But levels of nitrogen dioxide that are not rapidly fatal may cause more persistent ill effects, in some cases resulting in death from pulmonary edema after a delay of several days. [46] The same authors exposed albino mice at various concentrations of nitric oxide and measured the level of methemoglobinemia. [87] When mice were exposed to

nitric oxide at 2,100 ppm, 80% methemoglobin was produced in less than 30 minutes, 1,325 ppm nitric oxide produced 80% methemoglobin in about 40 minutes; and it required 6 hours of exposure to nitric oxide at 322 ppm to produce 60% methemoglobin.

The same authors exposed guinea pigs to nitric oxide at 175 ppm for 120-150 minutes and found no effect of this exposure on the rate of recovery of resting respiratory rhythm after treadmill exercise, compared with previous control measurements made on the same animals. [87]

In 1936, Zakusov [88] made a somewhat crude attempt to differentiate the effects of "nitrous gases" derived from heating nitric acid (predominantly NO2) from those associated with the action of copper metal on nitric acid (predominantly NO). Both gases were considered to be contaminated with nitric and nitrous acids. Experiments were performed on cats. The effects reported were similar to those of Flury and Zernik [86] and of Pflesser. [48] Zakusov [88] also observed that the gas derived from heating nitric acid produced more severe emphysema (as seen post mortem) following brief lethal exposures. The author based his diagnosis of emphysema on changes in lung weights, more likely indicative of pulmonary edema. He further concluded that nitric and nitrous acids may have contributed significantly to the findings.

Greenbaum and his colleagues, in an attempt to reproduce the conditions of an anesthetic accident, experimentally exposed dogs at lethal concentrations of nitric oxide and of nitrogen dioxide. [81] Either nitric oxide or nitrogen dioxide at a concentration of 5,000 ppm produced a rapid fall in arterial oxygen tension, a rise in methemoglobin concentration and a rise in arterial carbon dioxide tension, despite artificial respiration.

If exposure was continued for more than 24 minutes, all experimental animals died reportedly with overt pulmonary edema at intervals varying from 7 to 120 minutes after exposure. Nitric oxide at 20,000 ppm caused death in 15 to 50 minutes. It is interesting to note that in these animal experiments, methemoglobinemia was reported to be produced by nitrogen dioxide about as readily as by nitric oxide.

(b) Nitrogen Dioxide

There are many studies concerned with animal exposures to nitrogen dioxide or mixed oxides of nitrogen. In order to summarize as much of this information as possible, the data are grouped by concentration range, by type of effect, and by chronology. Greater emphasis is placed upon those concentrations and effects which appear to have some potential bearing upon the development of a standard for human occupational exposure.

(1) 50 ppm and over:

In an experiment designed to determine lethal concentrations in male rats for short-term exposures to nitrogen dioxide, Gray et al [89] found that the 2-minute LC50 (the minimal concentration killing 1/2 of the animals within 2 minutes) was 1,445 ppm. The 5-minute LC50 was 833 ppm, and for 15, 30, 60, and 240 minutes the corresponding LC50s were 420, 174, 168 and 88 ppm, respectively. The cause of death at all these concentrations was stated to be pulmonary edema, although no autopsy findings were reported.

In 1962, Carson et al [90] reported results of experiments on rats, rabbits, and dogs designed to determine the LC50 for short-term exposures (5-60 minutes) as well as concentrations which, for the same exposure times, did not result in macro- or microscopic changes in the

lungs, liver, kidneys, spleen, heart, eyes, and gastrointestinal tract. The LC50 values in rats were 416, 201, 162, and 115 ppm for 5, 15, 30, and 60 minutes of exposure, respectively. The rabbit LC50 for a 15-minute exposure was 315 ppm, considerably higher than that observed in the rat, ie, 200 ppm. Differences in the LC50 values for rats reported by Carson et al [90] and by Gray et al [89] were attributed by the former to differences in the size and age of experimental animals. However, the data of Carson et al [90] corroborated that of Gray et al [89] in showing that the product of concentration (C) and time (t) did not equal a constant (k), ie, $Ct \neq k$. Both studies indicated that the relationship between concentration and time was best represented by an exponential function. The thresholds for nitrogen dioxide toxicity were approximately 25% of the rat LC50 values. [90] At these concentrations, dogs showed no gross or microscopic changes which were different from controls. Rats showed no gross pathologic lesions; however, microscopic studies indicated that some animals had pulmonary edema.

In 1968, Kleinerman and Cowdrey [91] exposed 48 hamsters for 21-23 hours daily to nitrogen dioxide at 50 ppm for 1-10 weeks. Over one-third of the animals died within 2 or 3 days (7 on the first day, 6 on the second day). Other animals were killed at intervals. Microscopic examination immediately after cessation of exposure showed extensive epithelial hyperplasia and hypertrophy in the region of the terminal and respiratory bronchioles and alveolar ducts. Following 10 weeks of exposure, there were extensive focal collections of inflammatory cells and hyperplastic and hypertrophied epithelial cells in the same regions. While the size of the alveolar spaces appeared enlarged in exposed animals

compared with controls, there was no evidence of destruction of alveolar septal tissue. In animals killed 4 weeks after cessation of exposure, remarkable regression of inflammatory and epithelial hyperplastic changes were observed. Only a minimal degree of epithelial hypertrophy persisted in the respiratory bronchioles and alveolar ducts. There was no evidence of pulmonary edema, and acute inflammatory cells had virtually disappeared. These results indicated that the pulmonary lesions induced in the hamster under these exposure conditions were reversible. According to the authors, the results bring into question the belief that nitrogen dioxide, per se, causes true emphysema, ie, that due to tissue destruction.

(2) 5 to 50 ppm:

In 1952, Gray et al reported [92] experiments in which rats were exposed to the vapors of red fuming nitric acid, the airborne nitrogen dioxide content of which ranged from 9.3 to 14.3 ppm. The exposure schedule was 4 hours/day, 5 days/week. The total duration of exposure ranged from 10 to 24 days for different groups of animals. Rats examined shortly after the end of exposure showed severe rhinitis and tracheitis, with less severe pneumonitis. In many of the animals killed 8 or more weeks after the termination of exposure, the inflammatory process had subsided, but there were localized areas of emphysema in all lobes of the lung.

In 1965, Wagner et al [93] reported on the effects of exposure at 5 ppm on dogs and mice and of rabbits, guinea pigs, rats, and hamsters exposed at 5 and 25 ppm. The animals were exposed an average of 6 hours/day, 5 days/week, for periods ranging from 14 to 18 months. Dogs exposed at 5 ppm daily for 1 year showed only mild dilation of peripheral

air spaces. At 18 months, there was additional edema and congestion, accompanied by some thickening of alveolar septa. The respiratory tissues of the rabbits were essentially normal even after 18 months of daily exposures, at either 5 or 25 ppm. Hamsters exposed at 25 ppm daily for 3 and 6 months showed minimal evidence of changes in the bronchiolar epithelium. After 12 months, there was a questionably higher incidence of mild interstitial pneumonia. Exposure of two of the strains of mice used at 5 ppm daily for 10 and 14 months produced no observable microscopic changes or other abnormalities that could be attributed to the nitrogen dioxide exposure.

Diggle and Gage [94] exposed rats to nitrogen dioxide for single 4-hour periods at concentrations of 10, 22, 36, and 45 ppm. All animals had normal trachea and lungs 4-8 days after exposure. Kleinerman and Wright [95] subjected rats, guinea pigs, and rabbits to 2-hour exposures at 15-25 ppm for either 1 day or 5 days. Animals were killed at intervals of 1, 2, 4, 7, 14, and 21 days, and the lungs were studied macro- and microscopically. Twenty-four hours after a single 2-hour exposure, the animals' lungs showed a mild degree of pulmonary edema which was confined to the respiratory bronchioles. Macrophage infiltration and epithelial regeneration were noted by the fourth day and by the end of 2 weeks epithelial repair was almost complete. The degree of morphologic change and repair was roughly proportional to the concentration of exposure. Edema and inflammation were less severe in multiple exposures (5 days) than in single exposures. Tissue repair was almost complete 7 days after exposure. In general, peribronchial and perivascular inflammation was more severe in the rat and the guinea pig than in the rabbit. In 1962, the same

authors [96] reported results of experimental exposures of rats, rabbits, and guinea pigs at 20-25 ppm, 2 hours daily, 3-4 days a week, from 3 weeks to 18 months. The animals were killed 14 days after the completion of exposure. In 50% of the guinea pigs exposed for 15-18 months, changes judged to be equivalent to human microbullous emphysema were seen. Such changes were not, however, observed in rats or rabbits.

In 1964, Freeman and Haydon [97] presented data on rats exposed to nitrogen dioxide at 12.5, 25, 50, and 100 ppm continuously, ie, 24 hours/day, 7 days/week. At 100 ppm, the animals "began to die within 24 hours." At 50 ppm, 6 died within 48-68 days and 2 survived 76 days. At 25 ppm, all survived but failed to gain weight normally. Nine rats were exposed at 12.5 ppm. One died after 213 days, and autopsy showed pulmonary changes similar to those found in animals exposed at 25 ppm. In the surviving animals, those killed at about 40 days revealed moderate hypertrophy and hyperplasia of the bronchial and bronchiolar epithelium. The alveolar ducts and alveoli in exposed animals were more variable in size and many were much larger than those in controls. The animals exposed at 25 ppm who died between 146 and 157 days of exposure had "strikingly voluminous lungs." Haydon et al [98] exposed rabbits continuously at 8-12 ppm for 3-4 months. Various changes including emphysema-like dilatations of the peripheral alveoli were noted. In 1968, Freeman et al [99] reported results of the effects of continuous exposures at 18 ppm in rats. By the fifth day of exposure, hypertrophy of the terminal bronchial epithelium was seen, but no typical emphysema.

Riddick et al [100] continuously exposed mongrel dogs at 25 ppm for 6 months. One dog showed macroscopic bullous emphysema but all

showed bullous alveolar enlargement microscopically. Lewis et al [101] continuously exposed beagles at 26 ppm for 191 days. One dog showed bullous emphysema, and others showed "striking increase in firmness of the lungs with scattered small bullae." Emphysema was also noted microscopically.

The effect of continuous exposure at 15 ppm in rats was studied by Freeman et al. [102] Exposures were over the natural life time of the experimental animals. The animals were killed and found to have voluminous "dry" lungs (probably meaning nonedemetous) with large functional residual capacity. Microscopically, there was epithelial hypertrophy, "emphysema-like disease", and loss of cilia.

The significance of animal studies to man with respect to the production of emphysema or emphysema-like changes must be interpreted with caution. Tyler et al [103] have reviewed the comparative micro- and macroscopic anatomy of the terminal airways and air spaces and their blood supply in humans and in many of the common experimental animal species. On the basis of morphologic relationships, the authors concluded that the rat was an inadequate model for the study of human emphysema. According to the authors, the horse represented the animal model closest to man in terms of pertinent anatomical considerations.

Kilburn and Dowell [104] exposed mongrel dogs and rabbits to nitrogen dioxide at concentrations ranging from 5 to 16 ppm for 1 hour. Lungs of both species showed minimal microscopic changes consisting of perivenular edema without alveolar edema. Electron microscopy showed marked changes in the capillary endothelium and lesser changes in the alveolar epithelium. The endothelium formed blebs that encroached and

filled the lumens. Endothelial cell organelles, especially altered mitochondria, were found loose in capillary lumena. Platelets and polymorphonuclear leukocytes filled the lumena of involved capillaries adjoining blebs. Occasionally, platelets and leukocytes were found in edematous basement membranes. The authors concluded that brief exposure to nitrogen dioxide at low (nonlethal) concentrations has its major effects on capillary endothelium. Dowell et al [105] exposed beagles for 1 hour at concentrations of from 3 to 16 ppm. Electron microscopy revealed widespread bleb formation, loss of pinocytic vesicles, and mitochondrial swelling of endothelial cells. Exposure at only 3 ppm resulted in bleb formation in alveolar endothelium without biochemical or physiological changes.

Guinea pigs were continuously exposed at a concentration of 10 ppm for 6 weeks in a study conducted by Yuen and Sherwin. [106] An increased ratio of type 2 pneumocytes to other cells, resulting in thickening of the alveolar blood gas barrier, was observed. In 1973, Parkinson and Stephens [107] reported the results of an electron microscopic investigation of the lungs of rats exposed continously at 15 $\pm$ 2 ppm for 1, 2, and 7 days. Within 24 hours, loss of cilia was seen. The bronchiolar epithelium became less collumnar, brush cells increased in number, microvilli became smaller, and there was an increase in macrophages.

Sherwin et al [108] continuously exposed guinea pigs to nitrogen dioxide at 15 ppm for 3 months. (Because of an editorial error, confirmed by verbal communication with the senior author in September 1973, the term nitric oxide was erroneously substituted throughout this paper for

the correct term nitrogen dioxide.) Increased proliferation of alveolar cells with an increase in the alveolar cell/alveolar space ratio was demonstrated by a cytochemical technique which measured the lactic acid dehydrogenase activity of proliferating cells.

In 1968, Sherwin et al [109] reported increased macrophage congregation in the lungs and more macrophages per epithelial cell in guinea pigs following continuous exposure at 10 ppm for 7 weeks. Gardner et al [110] exposed rabbits for 3 hours at 8, 10, or 40 ppm. At 8 ppm, there was a significant increase in intra-alveolar heterophiles. At 10 ppm, fewer macrophages contained phagocytized bacteria. At 40 ppm, there was peak infiltration of heterophiles between 6 and 9 hours after exposure. The authors speculated that nitrogen dioxide exposure might destroy opsinogenic factors and surfactant, or in some way affect the motility of macrophages. Evans et al [111] continously exposed rats at 15-17 ppm for 48 hours. The results showed that there was a large increase in the number of dividing macrophages and an increase in the total number.

Buckley and Balchum [112] exposed guinea pigs continuously at 15 ppm for 10 weeks, and at 40 ppm for 1/2 hour every 2 hours for a total of 4 1/2 hours. Oxygen consumption of tissue homogenates (lung, liver, spleen, and kidney) was studied in vitro. Lung tissue oxygen consumption was minimally changed. Liver oxygen consumption was markedly increased following the acute exposure regimen. Lactic acid dehydrogenase (LDH) and aldolase activity were increased. Buckley and Balchum [113] exposed guinea pigs at 15 ppm, 23 hours/day, for 26, 33, or 40 days. A relative decrease in fast-moving (aerobic) isozyme and an increase in slow-moving (anaerobic) isozyme were detected in the lung only. In 1969, Buckley and Loosli [114]

reported on mice exposed continuously at 40 ppm for 6-8 weeks. An intense increase in LDH activity at the sites of nitrogen dioxide lung lesions was detected. The increase in oxygen consumption and in LDH activity suggested the stimulation of cellular activity.

Sherwin and Richters [115] exposed mice either continuously at 4-7 ppm for 14 days, or at 30 ppm for 24 hours. In all animals, leakage of tritiated serum into pulmonary lavage fluid was demonstrated, suggesting altered lung capillary permeability.

In 1965, Balchum et al [116] reported on guinea pigs exposed at 5 ppm for 4 hours/day for 5 days/week and at 15 ppm for 7 1/2 hours/day, 5 days/week, for 1 year. Only minor microscopic changes occurred but normal lung tissue serum antibodies appeared within 160 hours. The antibody titers increased thereafter with continued exposure. This is suggestive of an autoimmunization process triggered by the tissue destruction caused by nitrogen dioxide exposure.

Henry et al [117] in 1970 observed that squirrel monkeys exposed continuously at 5 ppm for 2 months and at 10 ppm for 1 month developed increased susceptibility to Klebsiella pneumoniae infection and to an influenza virus challenge given 24 hours before exposure. The authors [118] also reported that hamsters given single exposures at 15 ppm for 2 hours, followed by a 1-hour exposure to 3% volumn/volume (v/v) cigarette smoke showed decreased resistance to Klebsiella pneumoniae infection by enhanced mortality and decreased survival time. Valand et al [119] in 1970 observed that rabbits given a single 3-hour exposure at 25 ppm showed inhibition of production of interferon by their alveolar macrophages challenged by rabbit pox virus. Williams et al [120] conducted

experiments in which rabbits were given single 3-hour exposures at 25 ppm and then challenged with an influenza virus. More virus attached to nitrogen dioxide-treated macrophages than to controls. The inability of the nitrogen dioxide-treated macrophages to produce interferon was, therefore, not due to any defect in absorption, penetration, or uncoating of the virus. Acton and Myrvik [121] exposed rabbits to nitrogen dioxide for 3 hours at 5, 15, 25, and 50 ppm. Their virus-induced resistance and phagocytic activity were suppressed by 15 ppm. Fifty ppm stimulated oxygen uptake and hexose phosphate shunt activity of macrophages.

In 1970, Matsumura [122] reported investigations on the effect of nitrogen dioxide exposures upon antigen sensitization of the airways of guinea pigs. The animals were exposed at 20, 40, or 70 ppm for 30 minutes. Thirty to 50 minutes later, they were exposed to antigen aerosols for 45 minutes (egg albumin, serum, serum albumin). Exposure to nitrogen dioxide at 70 ppm enhanced sensitization via the airways; 40 ppm or less did not. In a later study, Matsumura et al [123] exposed guinea pigs to nitrogen dioxide for 30 minutes at 50 ppm, followed 30-50 minutes later by an exposure to acetylcholine aerosol. The latter exposure caused more severe respiratory distress in the nitrogen dioxide-exposed animals than in controls.

In 1964, Boren [124] investigated the possibility of synergism between nitrogen dioxide and carbon particles. One group of mice was exposed to nitrogen dioxide for 30 minutes/day, 5 days/week, for 4 1/2 months at a concentration of 25 ppm. A second group was exposed 6 hours/day, 5 days/week for 3 months to carbon with absorbed nitrogen dioxide, the latter having an airborne concentration of 25-30 ppm. The

combined exposure gave rise to focal and destructive lung lesions, apparently tantamount to emphysema although alveolar fenestrations were rare. No such findings were observed in animals exposed only to nitrogen dioxide at concentrations of 25 ppm. There was no evidence of fibrosis. In further experiments, Boren [125] observed that the sequence of carbon particulate and irritant gas exposures influenced the outcome. Inhalation of carbon alone (at 18,000-21,000 particles/ml) produced a macrophage response. Subsequent exposure to nitrogen dioxide at 25 and 75 ppm caused lung destruction. Inhalation of nitrogen dioxide followed by particulate carbon gave a lesser macrophage response and less lung destruction.

(3) 1.0 to 5.0 ppm:

In 1954, Gray et al [126] reported on exposing rats, mice, and guinea pigs 4 hours daily, 5 days/week for 6 months to vapors of red fuming nitric acid with an average airborne nitrogen dioxide concentration of 4 ppm. Not only did the animals exhibit no pathologic effects but they had a lesser incidence of pneumonia, from which the authors suggested a therapeutic effect of nitrogen dioxide.

House [127] continuously exposed rats, mice, and monkeys at an average concentration of 4.5 ppm. The mortality was low but all species were seriously depressed and weak with poor appetite and reduced weight gain. There were no other outstanding clinical or pathologic findings. This report has been contrasted with that of MacEwen and Geckler [128] in 1968 who exposed the same species at 5.0 ppm continuously for 90 days and found no significant mortality or any remarkable changes in blood chemistry or growth. Freeman et al [129] exposed rats at 2 ppm ($\pm$ 1 ppm) continuously for their natural lifetime. The rats showed persistent

tachypnea and all animals died of nonpulmonary diseases (predominantly of nephrosclerosis). Airflow resistance and dynamic compliance were not affected. Some microscopic changes were seen in the terminal and respiratory bronchiolar epithelium, ie, loss of exfoliative activity, reduced blebbing of cytoplasm into the airways, reduction or loss of cilia, and the appearance of rod-shaped intracytoplasmic crystalloid inclusions.

In 1972, Stephens et al [130] reported results of a study in which rats were exposed continuously at 2 ppm for periods ranging from a few hours to 21 days. They showed loss of cilia, hypertrophy, and focal hyperplasia in the epithelium of the terminal bronchioles. Animals allowed to survive returned to normal after 21 days of continuous exposure. Evans et al [131] exposed rats continuously at 2 ppm for up to 360 days. An increase in type 2 pneumocytes, ie, cuboidal rather than flattened and ultra-thin, was observed in peripheral alveoli after two days of exposure. However, the number of cells declined to control levels by the fifth day. There was no increase in cells in other alveoli or in the bronchioles. Sherwin et al [132] exposed guinea pigs at 2 ppm continuously for 1, 2, and 3 weeks. Replacement of type 1 pneumocytes (flattened and ultra-thin cells normally lining the alveoli) by type 2 pneumocytes in the alveoli was observed by a cytochemical technique based on LDH activity. A significant increase in the average area of each alveolar wall was also observed.

Chen et al [133] studied morphologic changes in the trachea and lungs of 15 mice exposed continuously for one month to nitrogen dioxide at between 1.0 and 1.5 ppm. The experimental design did not provide for the evaluation of control subjects. Animals were killed immediately following exposure and at intervals of 1 and 3 months following exposure.

Microscopic examinations revealed desquamative bronchitis in a number of animals in each sample group. Infiltration of lymphocytes was not observed in animals killed during or immediately following exposure. However, these effects were observed in 3 of 5 mice killed 3 months after exposure. The authors suggested that acute pathologic changes resulting from the exposures used in this study may lead to chronic autoimmune diseases such as chronic tracheitis or bronchitis in mice. It is difficult to assess the significance of these findings because of the inadequate controls employed.

In 1973, Arner and Rhodes [134] reported results of exposing rats at 2.9 $\pm$ 0.71 ppm for 24 hours/day, 5 days/week, for 9 months. A 12.7% mean increase in lung weights and 13% mean decrease of lung compliance were found at autopsy. There was also significant reduction in the surface-active properties of the lung-wash fluid.

Purvis and Ehrlich [135] exposed mice at 2.5 and 3.5 ppm for two hours followed by a challenge of Klebsiella pneumoniae. At the lower exposure level, there was no increased susceptibility to infection but at the higher level there was a significant increase in susceptibility for up to 27 hours after exposure. In 1970, Ehrlich et al [136] reported on single exposures of mice at 1.5, 2.5, and 3.5 ppm for two hours followed by respiratory challenge by Klebsiella pneumoniae. At the two lower levels of exposure, there was no significant increase in mortality. The threshold for this effect therefore appeared to lie in the region of 3 ppm nitrogen dioxide. Fenters et al [137] continuously exposed squirrel monkeys at 5 ppm for 169 days followed by a challenge by four intratracheal injections of a mouse-adapted influenza virus. Hemagglutination-inhibition antibody was not affected by nitrogen dioxide exposures. Initial production of

serum neutralizing antibody was influenced by gas exposure, but not after the 133rd day of exposure. In 1972, Ehrlich and Miller [138] reported results of in vitro exposures of Venezuelan equine encephalitis (VEE) virus to nitrogen dioxide at concentrations of 1.5, 5.0, and 10.0 ppm. At 5.0 ppm, the aerosol recovery and survival of VEE virus was significantly lower than in controls. Bacillus subtilis spores were exposed to nitrogen dioxide at 10 ppm but were unaffected. In 1973, Goldstein et al [139] reported on single exposures of mice at 1.9-14.8 ppm for four hours, and at 1, 2.3, and 6.6 ppm for 17 hours. The mice had been previously infected with radio phosphorus-labeled Staphylococcus aureus. With exposures above 7 ppm nitrogen dioxide, there was a progressive decrease in pulmonary bactericidal activity. Exposure at more than 2.3 ppm nitrogen dioxide prior to staphylococcal challenge also caused decreased bactericidal activity.

Coffin et al [140] studied the effects of intermittent and continuous exposures to nitrogen dioxide on mortality in mice challenged with Streptococcus pyogenes (Group C). Experimental animals were exposed on the basis of equivalent concentration-time products (Ct); for example, an exposure for 2 hours at 3.5 ppm would equal 7 ppm-hours. Results indicated that concentration was of considerably greater importance than time in determining the rate of mortality, ie, equal Ct's at different exposure times were not equally hazardous. Single exposures at 2.3 ppm for 3 hours and 1 ppm for 7 hours caused a slight but statistically insignificant increase in mortality from that of controls. The observation that mortality in mice exposed to nitrogen dioxide and then challenged with Streptococcus pyogenes does not follow a strict Ct=k relationship is

similar to that of earlier investigators [90,141] who came to similar conclusions based upon other criteria of effect. Cumulative effects of continuous exposure at 0.5, 1.5, and 3.5 ppm and intermittent (7 hours/day) exposure were also assessed by mortality figures. Exposure at 3.5 ppm resulted in a statistically higher mortality percentage than exposure at 0.5 ppm. In plotting the percent mortality as a function of exposure time, the linear regressions for exposures at 0.5 and 3.5 ppm were statistically significant at the 0.01 level, ie, from zero slope. The slope for the exposure at 1.5 ppm was significant only at the 0.25 probability level. Cumulative effects of exposure on mortality were also observed for intermittent exposures to nitrogen dioxide at 3.5 ppm. However, mortality as a function of total time of intermittent exposure was lower than that observed for continuous exposure. The authors' regression lines were from plots of mortality (linear) versus the logarithm of exposure time. Visual inspection of some of their curves suggests that a sigmoid relationship is a more likely regression than their linear regression. Thus, a plot of probit or logit mortality versus the logarithm of exposure time might have been more revealing.

Shalamberidze and Tsereteli [142] studied changes in the reproductive endocrine system of female albino rats exposed at 1.3 ppm, 12 hours daily, for 3 months. There was prolongation of the estrus cycle associated with an increased interestral period, a lengthening of the estral cycle, and a decrease in the monthly number of cycles. These changes became more pronounced with prolonged exposure. The capacity for pregnancy was not affected but the litter size and the fetal weights were decreased. This suggestion of alteration in endocrine or reproductive

function needs to be confirmed in other species and its relevance to humans then evaluated. If such influences pertain to humans, then special restrictions governing the exposure of female workers to nitrogen oxides would be indicated.

(4) 1.0 ppm and below:

In 1965, Wagner et al [93] reported on a series of long-term, intermittent exposures of 5 animal species to nitrogen dioxide at 3 different concentrations. Dogs, rabbits, guinea pigs, rats, and hamsters were exposed to nitrogen dioxide at 1 ppm for an average of 6 hours/day, 5 days/week for up to 18 months. Comparisons between experimental and control groups indicated no significant differences in percent body weight gain. Animals exposed daily for 6 months showed no microscopic reaction attributable to the inhaled gas. After 1 year, dogs showed a general pattern of moderately dilated alveolar ducts and sacs which contained some edema fluid and an occasional macrophage. Necropsy of dogs at 18 months showed, in addition to the above findings, an occasional area of mild to moderately thickened alveolar septa with chronic inflammatory cells.

Daily exposure of guinea pigs to nitrogen dioxide at 1 ppm for 3 months revealed an essentially normal lung parenchyma, a mild hyperplasia of bronchial epithelium, and a prominence of lymph follicles. At 3 and 6 months, the reaction in rabbits exposed daily at 1 ppm was that of an increased incidence of congestion with no visible lesions or other tissue alterations. The findings in the other 2 species exposed to nitrogen dioxide at 1 ppm were not described separately, but the authors implied that they were essentially negative.

In 1965, Haydon et al [143] reported on exposing rats

continuously at from 0.8 to 4 ppm for 16 weeks. No macroscopic emphysema was detected at autopsy, and only minimal microscopic changes were seen. In 1966, Freeman et al [144] exposed rats at 0.8 ppm continuously for their natural lifetime. The exposed rats showed a sustained tachypnea of about 20% above that of controls, but their growth and behavior were otherwise normal. Upon necropsy, only minimal morphologic changes were noted in bronchiolar epithelial cells. According to the authors, these changes were not accompanied by gross or microscopic obstructive diseases. Steadman et al [145] studied the effects on monkeys, dogs, rabbits, guinea pigs, and rats exposed at 0.5 ppm continuously for 90 days. The only effect reported was a possible slight weight loss. In 1969, Blair et al [146] studied mice exposed at 0.5 ppm for 6, 18, and 24 hours daily for 3-12 months. The expansion of lung alveoli was seen in all mice killed after 3-12 months of exposure, and the number of expanded alveoli increased with the duration of exposure. The general picture was interpreted as one of early bronchiolar inflammation with reduction of distal airway size and a concomitant expansion of alveoli. The overall lesions appeared to be consistent with the development of early focal emphysema. Kosmider et al [71] exposed guinea pigs at 1 ppm 8 hours/day for 180 days. They reported foci of emphysema and atelectasis, some bronchitis, bronchopneumonia, and extravasation of blood in the lungs. During the course of the exposures, the urinary excretion of hydroxyproline and acid mucopolysaccharides (possible degradation products of connective tissue) was increased. Total serum proteins, immunoglobulins, and weight gain were all diminished.

In 1974, Aranyi and Port [147] published the results of a study on the effects of continuous and intermittent exposure to nitrogen

dioxide on the respiratory defense mechanisms of mice. Separate groups of animals were exposed for 3 1/2 and 7 months either continuously at 2 ppm or intermittently (5 days/week) at 0.5 ppm with daily 1-hour peaks of 2 ppm (0.5/2 ppm). Other animals were exposed for 1, 3, and 6 months at 0.5 ppm presented continuously or at 0.1 ppm with daily 3-hour peaks of 1 ppm (0.1/1 ppm) presented intermittently (5 days/week). Experimental groups were matched by appropriate controls. Effects studied included phagocytic function, cell surface morphology, and oxygen consumption of the alveolar macrophages as well as terminal airway and alveolar morphology. Results indicated that cell counts, macrophage viabilities at isolation, and oxygen consumption of macrophages were unaffected by the experimental exposures to nitrogen dioxide. The in vitro phagocytic activity of macrophages in animals exposed for 3 1/2 or 7 months at 0.5 ppm nitrogen dioxide with 2-ppm peaks was significantly reduced compared with controls. However, exposure to nitrogen dioxide at 2 ppm nitrogen dioxide for the same time periods did not cause a change from control values. Examination of macrophages by means of scanning electron microscopy indicated significant morphologic changes in macrophages in those mice exposed at 0.5/2 ppm for 7 months. No changes in macrophage morphology were found in the other exposure groups. The lungs of animals exposed for 7 months at 2 ppm or 0.5/2.0 ppm as well as those of animals exposed for 6 months at 0.1/1 ppm showed changes in alveolar and terminal airway structures described as emphysematous.

In 1969, Vaughan et al [148] examined beagles exposed to nitrogen dioxide at 0.5-1.0 ppm plus nitric oxide at 0.2 ppm, 16 hours/day for 18 months. No differences in single-breath carbon monoxide diffusing

capacity, dynamic pulmonary compliance, or total pulmonary resistance were found in these animals in comparison with unexposed controls.

Bloch et al [149] studied effects on the cardiovascular system in beagle dogs exposed to automobile exhaust or to the components of automobile exhaust including two experimental exposures to combined nitric oxide and nitrogen dioxide, ie, low nitric oxide (0.204 ppm) with high nitrogen dioxide (0.52-1.04ppm) and high nitric oxide (1.53-2.04 ppm) with low nitrogen dioxide (0.208 ppm). Ninety-six dogs were exposed to filtered air or to 1 of 7 air pollutants for 16 hours/day over 4 1/2 years. Exposure procedures as well as methods used in the characterization of the exposure environment were not described. Two of 19 dogs in the control group showed abnormal static electrocardiograms (ECG's). One of these animals had what was described as a congenital mitral insufficiency. In the 11 dogs exposed to low nitric oxide-high nitrogen dioxide, 4 showed signs of abnormal ECG's or vectorcardiograms (VCG's). However, these findings may be questioned since complete measurements were not made on all animals, and corroborative evidence of cardiovascular abnormalities in these dogs was frequently absent, eg, one dog diagnosed as having pulmonic stenosis had normal ECG's in 5 out of 6 samples as well as marginally normal VCG's. Of the 11 dogs exposed to high nitric oxide-low nitrogen dioxide, none of the animals showed consistently abnormal static or postexercise abnormalities. The authors suggested that exposure to air pollutants could lead to the development of ECG and VCG abnormalities. However, statistical tests to confirm this inference were not performed. The ommission of procedural information coupled with the observation of congenital cardiovascular defects in control animals and incomplete or

inconsistent data in some dogs diagnosed as having abnormal cardiovascular function raises questions concerning the validity and significance of these findings.

In 1959, Ripperton and Johnston [150] reported on the exposure of rats at 0.15 and 0.5 ppm continuously for 2, 4, 5, and 6 weeks. No significant differences between experimental and control animals were detected in lung or liver tissues at necropsy. Blood catalase levels were higher in the exposed animals at five weeks exposure, but higher in the controls at six weeks. Excretion of glutamic acid and aspartic acid was higher in the exposed rats. Buell et al [151] observed spectrophotometric and microscopic changes of collagen and elastin in the lung tissue of rabbits exposed to 1 ppm for 1 hour. Animals killed immediately following exposure showed significant peak shifts in the protein absorbance spectrum as compared with controls. Preliminary microscopic evidence indicated some uncoiling of the 3-stranded twisted collagen fibers in exposed animals. Spectrophotometric peaks in the proteins of experimental animals killed 24-48 hours after exposure returned to the position of peaks exhibited by controls. The authors hypothesized that the shifts of absorbance spectra in exposed animals represented denaturation of collagen and elastin resulting from hydrolysis by nitrogen dioxide and consequent rupture of hydrogen bonds. Thomas et al [152] also exposed rats for 1 hour at 1 ppm, and for 4 hours at 0.5 ppm. The first exposure caused loss of cytoplasmic granules, disorientation, rupture, and reduction in the number of mast cells. The second exposure schedule led to degranulation of mast cells, predominantly around the mediastinal lung surface. These changes were reversible. Thomas et al [153] studied the effects of exposing rats at 1

ppm, 4 hours/day for 6 days. At necropsy, lung lipids, extracted by solvents, showed absorption spectra characteristic of diene conjugation, typical for oxidized polynoic fatty acids. Alpha-tocopherol (Vitamin E) pretreatment was only partially effective in preventing the lipid oxidation. In 1973, Sherwin and Carlson [154] reported that guinea pigs exposed at 0.4 ppm continuously for 1 week showed higher protein levels in the lavage fluid of the lung, indicative of protein leakage into alveoli.

Ehrlich [155] exposed mice at 0.5 ppm continuously for 3 months. The mice were challenged by airborne Klebsiella pneumoniae and an increase in mortality was observed as compared with controls. Mice exposed at 3.5 ppm for only 2 hours showed no statistically significant increase in mortality whether the nitrogen dioxide exposure preceded or followed a challenge by airborne Klebsiella. In 1968, Ehrlich and Henry [156] reported on exposures of mice at 0.5 ppm continuously for 3 months or for 6-18 hours daily for one year. The first schedule led to increased susceptibility to airborne Klebsiella with enhanced mortality. The second schedule led to increased mortality of mice and reduced capacity to clear viable bacteria from the lungs. Fenters et al [157] exposed squirrel monkeys at 1.0 ppm continuously for 493 days. The monkeys were then challenged five times with a monkey-adapted influenza virus. Only the exposed animals produced serum neutralization antibody within 21 days. Microscopic examination showed slight emphysema with thickened bronchial and bronchiolar epithelium.

Ehrlich et al [158] exposed Swiss albino mice continuously at a concentration of 2 ppm or intermittently (5 days/week) at 0.5 ppm with daily 1-hour peaks of 2 ppm (0.5/2 ppm). Animals comprising the control

group were exposed to filtered air. After 12 weeks of exposure, the mice were vaccinated with A2/Taiwan influenza virus vaccine. Exposures were continued for an additional 28 weeks, and animals were killed at intervals of 2, 4, 8, 12, 16, 20, 24, and 28 weeks. Measurements were made of hemagglutination-inhibition (HI), serum neutralization antibody formation (SN), and serum immunoglobulin levels. HI antibody titers declined at a similar rate for all animals over the course of exposure. SN titers were significantly decreased for mice exposed at 0.5/2 ppm of nitrogen dioxide as compared with controls. A similar difference was not reported between controls and animals continuously exposed to nitrogen dioxide at 2 ppm. Significant depressions of SN titers were also observed in animals exposed to filtered air for 12 weeks prior to vaccination and exposed at 2 or 0.5/2 ppm of nitrogen dioxide following vaccination. SN antibody titers measured beyond the 4th postvaccination week were not significantly different between the various groups. Serum immunoglobulin concentrations measured after 12 weeks of exposure indicated a significant decrease in immunoglobulin IgA and a significant increase in IgG1 levels in mice exposed to nitrogen dioxide. Concentrations of serum IgM and IgG2 were also higher in the nitrogen dioxide exposed samples; however, statistical significance could only be established for the 0.5/2 ppm group. Measurements of immunoglobulin levels (corrected for age), made on the 28th week following vaccination, indicated no significant differences between groups in terms of serum IgA concentration except for the group maintained in filtered air for 12 weeks before vaccination and exposed at 0.5/2 ppm after vaccination. These mice showed a significant increase in IgA. Concentrations of serum IgM, IgG1, and IgG2 were significantly elevated in

all mice exposed to nitrogen dioxide prior to or following vaccination as well as in mice continuosly exposed to nitrogen dioxide. An exception occurred in the group exposed continuously at 2 ppm nitrogen dioxide. Levels of IgG2 in this group were not significantly different from controls. The results of this study are difficult to interpret. First, it is difficult to assess the significance of the depression of SN antibody titers in animals exposed to nitrogen dioxide occurring at 2 weeks following vaccination in view of the similarities in titers between controls and NO2 exposed animals occurring beyond the 2nd week. Secondly, a number of the comparisons indicated that the 0.5/2 ppm exposures had a greater effect than the 2-ppm exposures on levels of immunoglobulins. This suggests that the degree of variability of the concentration or the peak may be important in determining immunoglobulin reactions in animals exposed to nitrogen dioxide. Finally, as the authors [158] recognized, although elevated levels of serum immunoglobulin have been identified with several chronic lung diseases, there are no data available reflecting cause-and-effect.

(c) Carcinogenesis and Mutagenesis

In 1965, Wagner et al [93] suggested that nitrogen dioxide might be tumor-promoting to the extent of causing an acceleration of the rate of appearance of lung adenomas in a spontaneous pulmonary tumor-susceptible strain of mice. Forty-nine mice were exposed at a concentration of 5 ppm, 6 hours/day, 5 days/week, for 12-16 months. After one year of exposure, there was a greater incidence of tumors in the exposed mice than in the control group (see Table XIII-11). After 14 months of exposure, the incidence of tumors was essentially the same for controls and exposed

animals. Experimentally exposed animals killed after 16 months of exposure showed the same number of pulmonary tumors as controls. Comments were generated as a result of these findings, criticizing the small sample size. [159] Although the incidence of tumors was greater in the exposed mice than in the controls following 12 months of exposure, subsequent statistical analysis shows this difference was not significant. In view of this statistical insignificance as well as the evident similarity in the incidence of tumors between controls and exposed animals after 14 and 16 months of exposure, the supposition that nitrogen dioxide has a tumor-promoting effect must be questioned. Further studies, with larger numbers of animals, should be performed before making inferences concerning the tumor-promoting capacity of nitrogen dioxide.

In a brief note with few supporting data, Henschler and Ross [160] reported that mice intermittently exposed to nitrogen dioxide at 40 ppm over a period of 18 months had no evidence of malignant tumors on serial section of lungs. They also found an increasing incidence of lung adenomas with a decreasing frequency of exposure, thus a decreasing dose. They added that there was not an increasing incidence of lung cancer in nitrogen dioxide-exposed workers, but gave no data in support of this conclusion. In 1968, Ross and Henschler [161] presented data on continuous exposures of hamsters to nitrogen dioxide at 40 ppm plus nitric oxide at 20 ppm for 16 months. Their results revealed no carcinogenic action of nitrogen oxides in the lung or in other organs. They reported the presence of adenomatous changes, but did not state whether there was an increase in test animals or provide data to allow a comparison with controls.

In 1973, Kuschner and Laskin [162] presented a summary progress

report on their current studies in pulmonary carcinogenesis. One hundred rats and 96 hamsters were exposed to nitrogen dioxide at approximately 25 ppm, 6 hours/day, 5 days/week, for up to 646 days. Carcinogenic findings were similar between exposed and control animals except for the appearance of an adenocarcinoma in one exposed rat. Kuschner and Laskin [163] also investigated the effects of combined nitrogen dioxide-benzpyrene (BP) exposure in rats. One group of 36 rats was exposed to fresh air while a second group of 36 rats was exposed to nitrogen dioxide at 25 ppm for 7 hours/day, 5 days/week. Thirty animals from each sample were exposed for 1 hour/day to a mixture of BP aerosol and nitrogen dioxide at concentrations of 10 mg/cu m and 18 mg/cu m (10 ppm), respectively. Preliminary results indicated the presence of squamous cell carcinoma in the lungs of one animal exposed to 25 ppm of nitrogen dioxide with repeated 1-hour exposures to combined nitrogen dioxide-benzpyrene. Similar qualitative changes in lung tissue have been observed in rats exposed to a combination of sulfur dioxide and benzo(a)pyrene. [164] However, complete results on nitrogen dioxide-benzpyrene exposures are not yet available.

Evidence of mutagenesis by nitrogen dioxide or its derivatives in animal species has not been found in the literature. However, nitrous acid, which is one of the products of the reaction of nitrogen dioxide with water at normal temperatures, [2] has been shown to have a potent mutagenic effect on lower forms of life such as the tobacco mosaic virus [165] and Escherichia coli. [166]

Correlation of Exposure and Effect

(a) Nitric Oxide

The effects of nitric oxide upon humans must be based upon inference from a limited number of animal experiments. It was interpreted by von Oettingen [45] that nitric oxide, per se, has no irritant properties, and that its principal direct action is to convert hemoglobin to methemoglobin. Again in the opinion of von Oettingen, [45] it is likely that the effects observed in nitric oxide poisoning are solely due to the hypoxemia which is secondary to the methemoglobinemia.

No quantitative exposure-effect relationships can be made for nitric oxide due to the absence of any measured environmental data on human exposures. The animal studies of Flury and Zernik [86] and Pflesser [48] show a dose-response relationship. Flury and Zernik exposed white mice to nitric oxide at 2,500 and 5,000 ppm. [86] It should be noted that the gas contained small amounts of nitrogen dioxide. At the higher concentration, the animals collapsed after 4-6.5 minutes and died after 6-8 minutes. At the lower concentration, collapse and death occurred after 6-7 minutes and 12 minutes, respectively. Animals removed to fresh air after 4 - 6 minutes' exposure recovered completely in a few hours to one day. In 1935, Pflesser [47] repeated these experiments in mice and observed marked cyanosis due to spectroscopically confirmed methemoglobinemia at 320-350 ppm. There was no macro- or microscopic postmortem evidence of pulmonary irritation, either clinically or microsopically, at necropsy.

Gray [80] made a direct comparison between Pflesser's report that 8 hours' exposure to nitric oxide at 310 ppm was nonfatal to mice and his own findings that the LC50 for rats after 4 hours' exposure to NO2 was about

1/4 of that level. Such a comparison is questionable considering the different animal species used and the different modes of nitrogen dioxide production employed in the two studies.

(b) Nitrogen Dioxide

The acute and usually delayed effects of higher concentrations of nitrogen dioxide on man are well established. After the initial response of irritant cough, which is often associated with mild headache, [41,49] and sometimes immediate but mild dyspnea, [23] there is a characteristic remission of symptoms for up to 12 hours before the onset of acute and potentially lethal pulmonary edema. [23,32,33,34,36,40,41,49] If the patient recovers from this first phase of pulmonary edema, there may be no further symptoms arising from the exposure incident. However, under certain circumstances which are probably related to the severity (total dose) of the exposure, an apparent relapse may occur. Relapses have been recorded for intervals ranging from a few days to several weeks following exposure. This takes the form of a second attack of acute dyspnea, cyanosis, cough, and fever which is usually more protracted than the first attack and may also be fatal. This symptom complex is thought to be due to an underlying pathologic lesion of the lung called bronchiolitis fibrosa obliterans. [34,41,44,50,51] The evidence suggests that, in most cases, if the patient survives this serious stage, total recovery takes place. [41,50,51]

The critical concentration of nitrogen dioxide needed to produce either acute pulmonary edema or bronchiolitis fibrosa obliterans is not known. There are considerable environmental data on sporadic human exposures to oxides of nitrogen which typically give rise to both acute

pulmonary edema and to bronchiolitis fibrosa obliterans. These conditions have not been reported at relatively steady low levels of exposure associated with those few industrial processes in which oxides of nitrogen are steadily generated and get into the workplace atmosphere. All reported cases have arisen as a result of sudden or intermittent emission of oxides of nitrogen from an accidental event such as an explosion or combustion of nitroexplosives, [44] the accidental escape or spilling of concentrated nitric acid, [34,37,53] the intermittent process of arc or gas welding, especially in a confined space, [52] or the imprudent entry into an agricultural silo which was not ventilated. [44,50,51] There are some environmental data available on the oxides of nitrogen and concomitant carbon dioxide in one farm silo filled with timothy grass, [29] but these were not related to any human exposure incident and merely indicate the potential for very high concentrations of nitrogen dioxide (up to 1920 ppm), nitric oxide (up to 630 ppm), and carbon dioxide (up to 60%).

Subacute and chronic responses to low levels of exposure to nitrogen dioxide are not well-established or defined in the human. For example, research has not definitively established that methemoglobinemia occurs in man through exposure to nitrogen dioxide, although there are theoretical considerations suggesting that it might occur. [167] In 1941, McCord et al [56] reported levels of methemoglobinemia very slightly above normal in 4 out of 14 welders exposed to "oxides of nitrogen" ranging from 2 to 10.3 ppm expressed in terms of nitrogen dioxide. The methemoglobin levels were 2.3% of the total hemoglobin in two welders, and 2.5 and 2.6% in the other two welders, barely above the physiological average normal of 1%. [84] In view of the analytical difficulties in estimating methemoglobin at that

period, [85] McCord et al's report [56] should be treated with some caution. In contrast, Morley and Silk [63] published in 1970 a study of shipyard exposures in arc and gas welders and cutters. Using a spectrophotometric method, they observed no abnormal level of methemoglobin in 22 men following a work-time exposure to oxides of nitrogen, reported as nitrogen dioxide, at 1-15 ppm.

From theoretical considerations, nitrogen dioxide would be expected to have an irritant effect both upon general mucosal surfaces and on the lower respiratory tract. However, the direct human evidence of such general mucosal surface effects is weak. A 1970 Russian study [64] reported a prevalence of 64% of chronic catarrhal processes in the upper respiratory tract in arc welders with up to 10 years' work history. In 22% of the welders with more than 10 years' service in welding, what was translated as subatrophic rhinopharyngitis was observed. However, these reported findings were not validated by comparison with any control group and there were no environmental data presented. In addition to oxides of nitrogen, arc welders are probably exposed to a variety of dusts and fumes from coated welding rods. Morley and Silk [63] reported acute or subacute conjunctivitis and pharyngitis in 5 gas welders who worked in a confined space on welding zinc-plated steel. All complaints had subsided 18 hours later.

There has been much speculation [71,97,98,99,102,143] whether chronic low-level exposure to nitrogen dioxide, among other irritant gas air-pollutants, may be a contributory cause of chronic obstructive pulmonary disease in man. Kennedy [28] in 1972 published a study of the prevalence of emphysema in 100 British coal miners repeatedly exposed to oxides of

nitrogen during underground blasting. Using pulmonary residual volume of more than 150% of normal as his diagnostic criterion, the author classified 84 of the miners as "probably suffering from an emphysema-like condition." Of these 84, 5 died during the period of the study and 4 of them had evidence of advanced generalized emphysema at autopsy. No definitive environmental data were given, but typical momentary concentrations of oxides of nitrogen following shot-firing were measured as high as 88 ppm. Misfires resulted in concentrations of up to 167 ppm. Graham and Runnicles, cited by Kennedy, [28] measured the concentration of nitrogen oxides in coal mines using black powder for shot-firing. Twenty-four-hour average concentrations at the coal face ranged from 0.8 to 4.5 ppm (nitric oxide + nitrogen dioxide, expressed as nitrogen dioxide), with 3-6.4 ppm in "hard headings." However, the high incidence of clinically diagnosed "probable emphysema-like condition" in coal miners could be attributed to the risk of coal workers's pneumoconiosis, which is known to be associated with focal emphysema. [73]

Kosmider et al [71] in 1972 reported an epidemiologic study on 70 chemical plant workers exposed 6-8 hours daily, for 4-6 years, to nitrogen oxides concentrations ranging from 0.4 to 2.7 ppm. There was evidence of chronic bronchitis in an unstated number of these workers. No abnormality was seen in the chest roentgenograms, and there were no significant changes in spirometric measurements or blood gases compared with controls. However, the total proteins in the blood serum were significantly lower than in controls. The exposed workers showed a reduction in albumin and gammaglobulins and an increase in the alpha-1, alpha-2, and betaglobulins. There were also significant increases in the urinary hydroxyproline and

acid mucopolysaccharides in the exposed men. The authors concluded that these abnormalities were indicative of protein synthesis inhibition and decomposition of collagen, consistent with the emphysema process.

Vigdortschik et al [70] published a study in 1937 on human exposures in sulfuric acid plants and etching operations in printing shops. The sample included 127 workers who had been exposed for 3-5 years to oxides of nitrogen at levels "mostly below 2.8 ppm." These exposures were thought to result in a number of symptoms and signs including dental erosion and gingivitis; emphysema and compensated pulmonary tuberculosis; hypotonia and bradycardia; and polycythemia rubra, granulocytosis, basophilia, and decreased osmotic fragility of erythrocytes. The evidence indicated a higher prevalence of the above conditions in the exposed workers than in carefully selected industrial worker controls, comparable in all respects except for nitrogen oxides exposure. However, the actual prevalence figures were not given. The statement that workers in sulfuric acid plants were exposed to nitrogen oxides and to no other injurious gases is questionable, especially in view of the finding of dental erosion, a well-known effect of sulfuric acid mist exposure. [168]

A small number of human experimental studies of the acute effects of low levels of nitrogen dioxide have been described. In 1967, Abe [67] reported a 40% decrease in the effective lung compliance of 5 healthy adult men 30 minutes after cessation of nitrogen dioxide exposure at 4-5 ppm for 10 minutes. Other indices of pulmonary mechanics were also altered. In 1971, von Nieding and Krekeler [68] reported increased airway resistance in 88 chronic bronchitis patients who had respired nitrogen dioxide-air mixtures containing as little as 1.5 ppm nitrogen dioxide, for either 15

minutes or for a total of 30 breaths. In 1973, von Nieding et al [69] found a statistically significant decrease in the carbon monoxide diffusing capacity of 16 healthy male volunteers following the inhalation of 5 ppm of nitrogen dioxide for 15 minutes. Patients with chronic bronchitis showed a significant increase in alveoloarterial pressure following inhalation of nitrogen dioxide for 15 minutes at 5 ppm. Increasing exposure time to 60 minutes did not alter alveoloarterial pressure from that observed during the first 15 minutes. It is not known whether any, or all, of these effects of acute low-level exposures in man are completely reversible following repeated intermittent exposures over a period of years.

Although the human studies have been helpful in delineating potentially harmful concentrations, a more scientifically rigorous approach to defining cause-and-effect relationships have been afforded by animal studies.

Much work has been done in attempts to produce emphysema in animals similar to that seen in humans by adjusting various schedules of exposure to nitrogen dioxide. Emphysematous conditions were produced in guinea pigs by Kleinerman and Wright, [96] in rabbits by Haydon et al, [98] in mongrel dogs by Riddick et al, [100] in beagles by Lewis et al, [101] and in rats by Freeman et al. [102] In all the foregoing experiments, the concentrations of nitrogen dioxide used were in the 5-50 ppm range. Few workers have claimed success in producing experimental emphysema with substantially lower concentrations of nitrogen dioxide. Blair et al [146] in 1969 reported "early focal emphysema" in mice exposed to 0.5 ppm for up to 1 year, while Kosmider et al [71] reported focal emphysema and other inflammatory lung changes in guinea pigs exposed to 1 ppm for up to 180 days.

Most researchers employing nitrogen dioxide concentrations below 5 ppm have observed subtle microscopic changes, such as reduction or loss of cilia, [99,130] hypertrophy and focal hyperplasia in the epithelium of the terminal bronchioles, [130] and a replacement of Type 1 (ultra-thin) by Type 2 (cuboidal) pneumocytes in alveoli, rather than emphysema. [131]

A problem in evaluating much of the animal studies is the imponderable relationship between the effects of continuous versus intermittent exposure. It might be assumed that for the same total dose of toxicant respired, intermittent exposure would be less harmful than continuous exposure at a correspondingly lower level. This assumption is made on the grounds that during the remissions of exposure the tissues have an opportunity to recover or repair themselves. The situation is further complicated in the case of nitrogen dioxide because it is the major constituent of the oxides of nitrogen which are, albeit at a low level, an air pollutant in most urban and heavily industrialized areas. Therefore, the typical industrial worker's exposure to nitrogen dioxide during the 14- to 16-hour daily respite from his industrial occupation will not be zero, but some fraction of his occupational exposure level. However, it is conceivable that for certain effects concentration of the toxicant might be the overwhelming determinant. Thus, for the same total respired dose, intermittent exposure might be more harmful than continuous exposure.

In view of the dose-response complexities of exposure to nitrogen dioxide, more weight should be given to those animal studies which have come as close as possible to an intermittent exposure schedule, those which parallel the typical occupational exposure schedule. One study, by Wagner et al [93] in 1965, reported the effects of exposing dogs and mice to

nitrogen dioxide at 1 and 5 ppm, and rabbits, guinea pigs, rats, and hamsters at 1, 5, or 25 ppm. All exposures were 6 hours/day, 5 days/week, for periods ranging from 10 to 18 months. Weight gain and blood studies including serum alkaline phosphatase activities were only minimally affected in dogs. Similarly, only minimal changes in the respiratory tissues of all animals were observed. The most frequently encountered pathologic alterations, a patchy or diffuse interstitial pneumonia and a type of chronic bronchiolitis, bronchitis, or bronchiectasis (with or without peribronchitis), were seen with equal frequency in the control animals. Moreover, there was no real difference between animals exposed at the three levels of 1, 5, and 25 ppm. These negative results contrast sharply with those of Freeman and Haydon [97] on rats, Haydon et al [98] on rabbits, Riddick et al [100] and Freeman et al [102] on rats. In all these studies, exposures were continuous to nitrogen dioxide at levels at or below 25 ppm. Reported pathologic findings included moderate hypertrophy and hyperplasia of bronchial and bronchiolar epithelium in rats, [97] emphysema-like dilatations of the peripheral alveoli in rabbits [98], bullous alveolar enlargement in dogs, [100] and loss of cilia, epithelial hypertrophy, and "emphysema-like disease" in rats. [102]

Exposure at concentrations of nitrogen dioxide of 0.5 ppm may also increase susceptibility of animals to subsequent infection by Klebsiella pneumoniae or Streptococcus pyogenes. [140,155,156] This phenomenon has also been observed at somewhat higher concentrations of nitrogen dioxide (1-5 ppm) [135,136] and at concentrations in the 5-to-50 ppm range. [117,118] At these higher concentrations, decreased resistance to subsequent challenge by certain viruses [117] and inhibition of interferon

production have been reported. [119,120] Although this effect has potential human implications, no such phenomenon has been clearly described in man. The secondary bacterial invasion and ensuing bronchopneumonia of severe cases of pulmonary edema or bronchiolitis obliterans following high exposures to nitrogen dioxide are probably due to the gross tissue damage and loss of integrity of epithelium rather than to interference with immunologic or phagocytic mechanisms.

A variety of other effects of nitrogen dioxide exposures including changes in pulmonary physiology, biochemical changes in the lung, pulmonary surfactant, enzymatic changes in remote organs, changes in macrophage populations and behavior, immunologic changes, etc, have been described in this chapter but have not been reviewed again here as they do not appear to have a meaningful bearing on the development of an occupational environmental standard.

The question of carcinogenicity or mutagenicity of the oxides of nitrogen in man will require evidence before any conclusions can be made. Wagner et al [93] exposed a strain of mice susceptible to spontaneous pulmonary tumors at moderate concentrations of nitrogen dioxide and reported that such exposures accelerated the rate of tumor development. This evidence is quite tenuous since statistical comparisons between exposed and control animals showed that the differences were not significant and the final incidence of tumors was unaffected. Other long-term exposure studies of mice [160] and of hamsters [161] reported the production of adenomatous changes, but they failed to find any evidence of malignant lesions. The possibility exists that nitrogen dioxide may act as a cocarcinogen or carcinogenic "promoter"; however, data are insufficient

to support this contention at this time. Nitrous acid has been shown to be a potent mutagen for viruses [165] and for bacteria [166]; however, evidence that nitrogen dioxide and nitric oxide are mutagenic in humans has not been found.

IV. ENVIRONMENTAL DATA

Environmental Concentrations

In 1940, Case and Castrop [17] measured the oxides of nitrogen (reported as nitrogen dioxide) at various distances from welding arcs under different conditions of ventilation. Thirteen samples were collected in a large unventilated room. Measurements were made 3 inches from the electric arc. Results indicated concentrations of nitrogen dioxide ranging from 30 to 70 ppm. However, they reported finding no nitrogen dioxide at a distance of 18 inches from the arc. Samples of a variety of mechanically ventilated electric welding operations involved in automobile manufacturing were also collected. Most of these showed 1 ppm or less of nitrogen dioxide. Two samples taken 12 inches from the arc showed 4 ppm, and one, 18 inches from the arc, showed 11 ppm. Five samples collected between 2 and 6 inches from an acetylene welding flame produced nitrogen dioxide concentrations ranging from 6 to 40 ppm. Only traces were found 18 inches from the flame. In the opinion of the authors, none of the methods then available for estimating nitrogen dioxide in air were entirely satisfactory. They used the alpha-naphthylamine and sulfanilic acid method (see Environmental Sampling and Analytical Methods in this chapter).

Adley, in 1946, [11] reported on nitrogen oxides produced by oxyacetylene torch shrinking operations in ship construction. Nitrogen oxides at an average of 196 ppm with a maximum of 480 ppm were obtained from eight samples collected over a 23-minute period in a 600-cu ft unventilated compartment. The torch had a rated capacity of 125 cu ft of acetylene/hour. Further tests showed that the rate of nitrogen oxides

production was directly related to the rate of acetylene consumption. An average of 38 ppm of nitrogen oxides was produced in a 700-cu ft compartment with an acetylene consumption of 15.9 cu ft/hour. There was a consistent increase up to 352 ppm of nitrogen oxides when acetylene consumption reached 175 cu ft/hour. Adley also made measurements in large, ventilated compartments with areas ranging from 7,200 to 10,000 cu ft. Concentrations found in 28 samples varied from 4 to 89 ppm. The phenoldisulfonic acid method was used for sample analysis. [169] Details of Adley's findings are given in Tables XIII-12 and XIII-13.

Dreessen et al [13] reported results of over 2,000 samples collected at a variety of welding operations in seven shipyards. Approximately 43% of the samples exceeded 4 ppm. Analysis was by the phenoldisulfonic acid method. Further data are presented in Table XIII-14.

Mangold and Beckett in 1971 [18] presented data on samples collected at silver brazing operations. Measurements were made following the hospitalization of 2 silver brazers from a naval shipyard. Exposure to cadmium oxide fumes was suspected as the principal cause of their acute pulmonary distress; however, tests revealed that no cadmium-bearing solders were used. Samples of nitrogen dioxide indicated that concentrations quickly reached a level of 50 ppm and increased to 122 ppm in 30 minutes.

Surveys on plasma torch operations by various investigators [12, 21,170,171] reported nitrogen oxides concentrations from "not detected" to more than 50 ppm.

Kosmider et al, [71] in a study of men from a chemical works, reported that these workers were exposed daily at nitrogen oxides concentrations fluctuating between 0.4 and 2.7 ppm as nitrogen dioxide over 6-

8 hours. No details were given as to the nature of the operations or to the methods employed in determining the air levels.

Formation of N-nitroso Compounds

The oxides of nitrogen have the intrinsic chemical property of reacting with primary and secondary amines which are present in body tissues, [172] foods, [173] tobacco, [173] water, [174] and in workplace air of some occupational environments [175] to form N-nitroso compounds. The nitrosation of primary amines produces unstable compounds which can decompose to form diazonium salt derivatives while the nitrosation of secondary amines produces secondary nitrosamines. [176] The metabolic dealkylation of secondary nitrosamines can lead to the formation of unstable diazonium salt derivatives. [176] Alkylated derivatives of proteins, nucleic acids, and other cellular constituents have been isolated following the in vivo treatment of mammals with secondary nitrosamines or the in vitro treatment with diazonium salt derivatives. [176] Subcutaneous, intravenous, intraperitoneal, percutaneous, and oral administration of secondary nitrosamines in experimental animals has resulted in the formation of tumors in several different organs. [176] The rate of tumor formation in rats following administration of secondary dialkylnitrosamines has been extensively investigated by Druckrey [177] who reported a linear-logarithmic relationship between the daily dose and the induction time for several dialkyl nitrosamines.

The reaction between secondary amines and the oxides of nitrogen is catalyzed under acidic conditions [178] as may be found in human gastric fluids or in the presence of acidic atmospheric contaminants. Although the

optimum pH for this reaction lies in the range 2.0 to 3.4, [178] formation of nitroso compounds can occur under neutral conditions. [174] Data on the in vivo formation of nitrosamines have been extensively reviewed by Mirvish [178] in 1975. For the most part, these studies have been limited to the investigation of intragastric formation of nitrosamines in animals who ingest oxides of nitrogen and secondary amines or in animals given the two compounds by gavage. The formation of nitrosamines is dependent upon a number of factors including the structure of amine, pH, order of administration of the two compounds, absorption of nitrite in the stomach, dose, and the presence of nitrite-competing compounds such as ascorbate which deplete the supply of available nitrites. [178] Experimental data on the in vivo formation of nitrosamines following inhalation of the oxides of nitrogen have not been found in the literature. The observations of Henschler and Ross [160] and Kuschner and Laskin [162] do not indicate production of carcinomas in mice, rats, and guinea pigs intermittently exposed to nitrogen dioxide for 1 1/2 to 2 years at 40 ppm and 25 ppm, respectively. However, further research should be conducted to determine the rate of in vivo formation of secondary nitrosamines following inhalation of oxides of nitrogen at low concentrations, and the possible effect of such in vivo formation on the induction of neoplastic changes.

Of particular concern is the airborne formation of secondary nitrosamines in the occupational environment. In 1973, Bretschneider and Matz [175] reported measuring secondary nitrosamines in the workplace air of plants involved in the production of dimethyl- and diethylamines. Samples, collected on activated charcoal, were eluted by diethyl ether and analyzed by gas chromatography. Levels of dimethylnitrosamine (DMN)

measured in one plant manufacturing dimethylamine ranged from 0.001 to 0.43 parts per billion (ppb). Samples collected over a period of 3 days indicated a decline in the airborne concentration of DMN to nondetectable levels by the third day. Airborne levels of nitrogen dioxide were found to decrease to 1/2 of the initial concentration over the same sampling period. The correlation between the airborne concentrations of DMN and nitrogen dioxide is interesting in light of data reported by Mirvish [179] indicating that the rate of DMN formation in buffered aqueous solutions was proportional to the concentration of dimethylamine and to the square of the nitrite concentration. However, the unexplained finding that the DMN could not be detected in the workplace air on the third day of measurement despite of 10-fold increase in the concentration of dimethylamine raises serious questions about the reliability of the sampling method.

Fine et al [180] reported data indicating the presence of N-nitroso compounds in the air near chemical plants in two urban communities. Air was sampled through three successive cold traps at a rate of 1.8 liters/min. for 2 hours. Analysis was by means of a gas and liquid chromatograph interfaced with an N-nitroso specific Thermal Energy Analyzer. Of five urban areas selected for analysis, two had detectable levels of dimethylnitrosamine. Concentrations of DMN near a dimethyl hydrazine plant in Baltimore ranged between 0.033 ppb and 0.96 ppb. Only trace levels of DMN were detected in samples taken during nighttime operations. [Fine, as cited in 181] This finding is surprising since the concentration of N-nitroso compounds is presumably higher under low ultraviolet light exposure. [175,177] Since DMN is a precursor for dimethyl hydrazine, the levels measured in Baltimore could represent leakage of DMN

into the atmosphere rather than in airborne formation of the compound. However, airborne formation is suggested by the results of measurements made near a dimethylamine plant in Belle, West Virginia where concentrations of DMN ranged between 0.014 ppb and 0.051 ppb. Collectively, the work of Bretschneider and Matz [175] and Fine et al [180] suggests that N-nitroso compounds may be formed in the workplace air or in the sampling train. However, extensive research will be required to validate sampling and analytic methods for measuring volatile N-nitroso compounds. Additional research should be conducted to: (1) measure and verify the levels of N-nitroso compounds in the air of occupational environments where secondary amines are produced, (2) determine the rate of formation of secondary nitrosamines in the workplace air, and (3) identify the environmental factors influencing the formation and decomposition of secondary nitrosamines in the occupational environment.

Isolated experimental studies indicate that the inhalation of secondary nitrosamines is acutely toxic in laboratory animals. Jacobson et al [182] exposed groups of rats, mice, and beagle hounds for 4 hours to nitrosodimethylamine vapor at various concentrations between 16 and 188 ppm. The LC50 values for rats and mice exposed to nitrosodimethylamine for 4 hours and observed for 14 days were 78 ppm and 57 ppm, respectively. Although the LC50 for beagle dogs was not determined, the dogs appeared to succumb at lower concentrations than did the rats and mice. Effects noted included polydypsia, loss of appetite, ascites, hyperacute liver necrosis, leukopenia, disruption of normal blood coagulation, and hemorrhage into the wall of the gastrointestinal tract, the abdominal cavity, and other tissues. Analogous effects have been noted in humans accidentally exposed

to nitrosodimethylamine at unknown concentrations. [183] Druckrey et al [184] studied neoplastic changes occurring in rats exposed either once or intermittently (1/2 hour/week) to dimethylnitrosamine or methyl vinyl nitrosamine. The 1-hour LC50's for dimethylnitrosamine and methyl vinyl nitrosamine were 506 ppm and 203 ppm, respectively. Of 12 rats exposed once/week for 1/2 hour to 50 ppm of dimethylnitrosamine, 9 died of carcinomas which developed from the ethmoid cells after 260-450 days of exposure. Similar exposure to methyl vinyl nitrosamine resulted in the death of 6 out of 12 rats after 230-330 days of exposure. Unlike dimethylnitrosamine, exposure to methyl vinyl nitrosamine produced carcinomas of the epithelium in the anterior nasal cavity. Dontenwill et al ([185] observed the development of tumors in the trachea and lungs of golden hamsters exposed to diethylnitrosamine aerosol (1-2mg) twice weekly. After 5 months of exposure, 18 out of 33 experimental animals had carcinomas of the trachea, lungs, or of both. The remaining 15 animals showed metaplastic changes in these areas. Similar exposures in 10 rats did not result in tumors after 3 months of exposure. However, the concentration of exposure in the rat experiments was not given.

In summary, experimental animal studies indicate that inhalation of relatively high concentrations of secondary nitrosamines for short periods of time can result in liver damage and severe hematologic changes as well as in carcinomas of the nasal cavity, trachea, and lungs. Jacobson et al [182] and Dontenwill et al [185] have observed widely varying sensitivities in nonprimate species exposed to airborne nitrosamines. Except for the ill-defined exposure used by Dontenwill et al, [185] the animal studies which have been reviewed have used exposures at concentrations which are

approximately 1 million times greater than that reported in the occupational environment. No animal data have been found on the chronic effects of long-term exposure at low airborne levels of nitrosamines, or on the in vivo formation of N-nitroso compounds from inhalation of the oxides of nitrogen. Furthermore, no epidemiologic data have been found indicating chronic effects in humans exposed to airborne secondary nitrosamines. Serious questions yet remain on the reliability of sampling techniques used to monitor nitrosamine levels in the working environment. Therefore, although a potential human health hazard exists from the airborne or in vivo formation of N-nitroso compounds, the currently available data base is insufficient to establish the degree of risk to occupationally exposed workers.

Control of Exposures

Since nitrogen oxides can be produced under a variety of situations, the appropriate engineering controls will vary. For welding and related types of operations, local exhaust ventilation, located as close as practicable to the source of heat, is needed. Because the nature of the work may require the welder to be frequently changing the location of operations, the exhaust system must be mobile. This may necessitate the use of flexible exhaust ducts or other arrangements which would place the hood or duct opening at the most effective location. [186,187]

Operations, such as bright dipping of metals with nitric acid, conducted in stationary tanks lend themselves to conventional local exhaust ventilation practices. [186,188] Exhaust systems must be resistant to acid gases. In a laboratory pilot study, Kerns [189] observed that the release

of nitrogen oxides from bright dipping and similar operations can be effectively suppressed by the addition of urea to the acid in the tank; however, the practice has not gained general acceptance in operational procedures.

Control of nitrogen oxides from indoor vehicular operations is usually dependent upon provision of sufficient general ventilation. Where work is being done upon stationary vehicles as in repair garages, local exhaust systems should be attached to or located close to the vehicle tail-pipes to limit exposure not only to nitrogen dioxide, but to other contaminants as well, particularly carbon monoxide. Similar considerations are applicable to gas and diesel engines operated in enclosed areas, such as mines and tunnels.

Design principles contained in publications on ventilation systems [186,187,188] should be followed if exhaust ventilation is needed for the control of nitrogen oxides.

Where blasting is done in tunnels or mines, workers should not be permitted back into the area until the ventilation system has had time to clear out the blasting gases.

The hazard from oxides of nitrogen in agricultural silos is greatest during the first week to 10 days after green ensilage has begun fermenting. [29,190,191,192] Workers should not enter silos which have not been properly ventilated. Wherever there is a possibility that the concentrations of oxides of nitrogen, of other contaminants, or of oxygen are not safe, workers should enter a silo with an acceptable respirator which should be, and if oxygen is deficient must be, an air-supplied type.

Additional personnel should be available for rescue operations.

Environmental Sampling and Analytical Methods

Several instrumental methods for measuring nitrogen dioxide have been suggested. Morrison et al [193] and Morrison and Corcoran [194] have reported on nitrogen dioxide determinations by electron-capture detection in gas chromatography. Techniques for obtaining breathing zone samples in the occupational environment and for the transport and preparation of samples for analysis by such a procedure remain to be worked out. Coulometric instruments [195] also represent a possible measurement method. This method is based on the reduction of nitrogen dioxide by bromide or iodide with the release of bromine or iodine, respectively. Bromine and iodine produce a current which may then be measured by a microammeter. Alternatively, voltage drop may be measured. Guicherit [196] observed that the efficiency of a specific coulometric instrument is low and the reactions not specific for nitrogen dioxide. In 1972, he reported a chemiluminescent method for measuring nitric oxide for application to air pollution studies. Commercially produced instruments are now available. Nitrogen dioxide may be determined by a chemiluminescent reaction between oxygen atoms and nitrogen dioxide, or by indirect means. In the latter method, ozone, produced by nitrogen dioxide photolysis, is measured utilizing a chemiluminescent reaction between ozone and an organic compound. Size and portability factors make the use of such equipment unsuitable for measuring breathing zone exposures. The method probably could be used for area monitoring. The equipment cost is considerable, and the complexity of the method requires highly trained personnel for

operation and maintenance.

Early industrial hygiene studies of total nitrogen oxides exposures employed the phenoldisulfonic acid method. [169] The procedure consists essentially of the absorption of nitrogen oxides in an acid solution to form nitrous and nitric acids, followed by oxidation with hydrogen peroxide of the nitrous acid to nitric acid. Potassium hydroxide is added, and following evaporation to dryness, the nitrates are treated with phenoldisulfonic acid, forming a yellow color. The intensity of the color is proportional to the concentration. Presumably, the method can be made specific for nitrogen dioxide by passing the air sample through silica gel, which absorbs the nitrogen dioxide, but not for nitric oxide. [197] However, Wade et al [5] found that nitrogen dioxide is not absorbed by silica gel but is converted to nitric acid. Although the phenoldisulfonic acid method is exacting and time-consuming, it is very sensitive. [198,4]

Patty and Petty [199] developed a field technique using sulfanilic acid and alpha-naphthylamine (Greiss-Ilosvay reagent). This technique uses a 50-ml hypodermic syringe into which the reagent is placed before the sample is collected. The color developed is compared with standards. A kit employing this procedure is commercially available. However, since the procedure is for nitrites, [199] it is nonspecific for nitrogen dioxide or nitric oxide.

In 1954, Saltzman [4] reported on screening tests for absorption efficiency performed on a number of reagents. He developed a modification of the Greiss-Ilosvay reagent which is specific for nitrogen dioxide. The method, which has been widely used for both community air pollution (40 CFR Part 50) and occupational exposure studies, [18,19,29] uses sulfanilic acid

and N-(1-naphthyl)-ethylenediamine dihydrochloride. Automatic continuous recording equipment, as well as grab sampling devices such as evacuated bottles or syringes, are used with the Saltzman reagent. [200] Length-of-stain detector tubes using this reagent have also been developed. [197,201,202] Detector tubes, while only semiquantitative, [201] can be valuable adjuncts to the compliance method, especially for the determination of exposure areas and for special purposes of identification of hazardous conditions. The use of detector tubes, while not as sensitive or precise as the compliance method described in Appendix I, does have the advantage of simplicity and of giving results immediately. A description of the use of detector tubes is given in Appendix III.

Most analytical procedures for nitric oxide involve oxidizing it to nitrogen dioxide and subsequently determining the concentration of that compound. [5,201,203,204,205,206,207] Consideration must be given to the fact that some nitrogen dioxide is also present in occupational or environmental exposures. [198,4,5, 6,9,14,16,23,10,208] In most published investigations of occupational exposures, all of the mixtures of oxides have been oxidized to nitrogen dioxide, and only the totals reported. If each is to be reported separately, one of two basic approaches is usually employed. The nitrogen dioxide portion of the sample may be separated out and measured, with the nitric oxide in the remainder then being oxidized to nitrogen dioxide and determined by the same procedure. The alternative approach is to collect two samples simultaneously at the same location (or to split one sample into two portions). One is analyzed for nitrogen dioxide, the other for total nitrogen oxides, with the nitric oxide being

the difference. [204,205,207,209]

Extensive work has been done to improve methods for precise determinations of airborne concentrations of nitric oxide. [204,205,206,209] Much of this work has been incorporated into a tentative method for nitric oxide recommended by a chemical standards setting group, known as the Intersociety Committee. [207] In this method, nitrogen dioxide is first removed by a solid adsorbent containing triethanolamine. After adjustment of the humidity of the remaining sample, it is passed through an oxidizer made of chromium trioxide deposited on a solid material. The nitric oxide is then converted to nitrogen dioxide, after which it enters a bubbler containing Saltzman reagent. The absorbance is then read at 550 nm. The Saltzman method is a sensitive colorimetric procedure. All of the reagents are incorporated in one solution, which is an efficient collection medium for nitrogen dioxide. The color is partially developed during sampling, which is advantageous since the sampling time can be adjusted to provide adequate color for accurate photometric measurement. [210]

Blacker [211] reported on a field method to sample nitrogen dioxide in the range of concentrations encountered in the work environment. The sample is collected on a triethanolamine-impregnated molecular sieve surface, [212] using the principle described by the Intersociety Committee. The solid-type adsorber is simpler to use and avoids other disadvantages encountered in trying to perform personal sampling with a liquid-type collector. In addition, Blacker reported that samples collected on the solid adsorber have a high degree of stability for at least 2 weeks. This

is important in the event that analyses cannot be performed promptly.

If a second solid-type adsorber is substituted for the bubbler in the sampling train described by the Intersociety Committee, a personal sampling device for measuring both nitrogen dioxide and nitric oxide is produced. Such a device, described in Appendix I, represents the recommended method for sampling the oxides of nitrogen. The method is recommended because of its simplicity, suitability for personal sampling, and because samples remain stable during shipment to the laboratory.

The samples may then be analyzed by the procedure described by Blacker [211] and by Levaggi et al. [212] This procedure is described in Appendix II. Since, in the sampling procedure, the nitric oxide is converted to nitrogen dioxide, the same analytical procedure serves to determine both substances.

Tests of the recommended sampling train by NIOSH [213] indicated that recovery rates for nitrogen dioxide were greater than 90% for samples generated at concentrations of between 0.8 and 5.6 ppm. The average recoveries for nitric oxide were 97.4, 106, 84, and 67% for sample concentrations at 8.6, 11.0, 24.0, and 50.0 ppm, respectively. The total coefficient of variation was 7.2% for nitrogen dioxide and 5.5% for nitric oxide.

V. DEVELOPMENT OF STANDARD

Basis for Previous Standards

On the basis of results of studies conducted with cats and rabbits, Lehmann and Hasegawa [39] stated that a concentration of oxides of nitrogen equivalent to 0.1 mg/liter of nitrous and nitric acids, calculated as nitric acid, could be withstood by humans for several hours. This is equivalent to 39 ppm of nitric acid at 25 C and 760 mmHg pressure. According to Schrenk, [214] this figure of 39 ppm was suggested for a number of years as a Maximum Allowable Concentration (MAC) for the oxides of nitrogen by several different authors and agencies in the US.

In 1937, a MAC of 10 ppm as nitrogen dioxide was suggested as an guide to manufacturers in the state of Massachusetts. [214] In a then comprehensive listing of MAC's, published in 1945, Massachusetts was the only state to list a MAC for nitrogen dioxide of 10 ppm. [215] For the states of California, Connecticut, and Oregon, and for the US Public Health Service and the American Standards Association, the MAC was 25 ppm for "nitrogen oxides." For the states of New York and Utah, MAC's of 10 and 10-40 ppm "nitrogen oxides" were recommended, respectively. As documentation for the 25 ppm MAC's, Cook [215] cited the animal experiments of La Towsky et al [216] who concluded that 3-hour exposures to oxides of nitrogen at 30 ppm produced no immediate or delayed harmful effects in guinea pigs.

In 1946, the American Conference of Governmental Industrial Hygienists (ACGIH) [217] recommended 25 ppm as the MAC for "nitrogen oxides (other than nitrous oxide)." In 1948, the ACGIH numerical recommendation

remained unchanged, but the term Threshold Limit Value (TLV) was substituted for MAC.

Gray et al [92] reported results of exposure of rats to the vapors of red fuming nitric acid, the nitrogen dioxide content of which ranged from 9 to 14 ppm. They found evidence of severe pulmonary congestion, bronchiolitis, and pneumonitis following exposures for 4 hours/day, 5 days/week, for a total exposure of 40 - 96 hours. On this basis, they [92] commented that the TLV of 25 ppm recommended at that time was too high. In 1954, Gray et al [126] published the results of a study on rats exposed on the same schedule for 6 months. The concentration of vapors from red nitric acid was 4 ppm. No toxic effects were observed. As a result, they recommended that the MAC for the oxides of nitrogen be set at 5 ppm. In 1954, the ACGIH [218] reduced their recommended TLV to 5 ppm, and, at the same time, changed the designation from "nitrogen oxides (other than nitrous oxide)" to "nitrogen dioxide." According to the ACGIH 1971 Documentation of the Threshold Limit Values, [219] the reduction to 5 ppm was based upon the results of the work of Gray et al in 1952 and 1954. [92,126]

During the period from 1948 to the mid-1950's, the 8-hour TWA concept of the TLV had evolved. In 1964, the ACGIH established 5 ppm as their recommended ceiling value for nitrogen dioxide, instead of an 8-hour TWA. [141] The basis for this more stringent recommendation was the report of Wagner et al [93] published later in 1965, which suggested a possible lung tumor accelerating capacity of nitrogen dioxide for spontaneous lung tumor-susceptible mice. [219] It was believed that by imposing this ceiling of 5 ppm, thereby effectively reducing the TWA exposure to less than 2.5 ppm,

the risk of accelerating lung tumor development in man would be minimized. [219]

The American National Standards Institute (ANSI) recommended a 5-ppm ceiling as acceptable for repeated daily exposures to nitrogen dioxide. [220] A concentration of 15 ppm was defined as the maximum "peak" for an 8-hour workday. The total exposure period at this "peak" was not to exceed 5 minutes and it was assumed that such excursions above the ceiling would be infrequent (not daily).

The present federal standard for nitrogen dioxide is 5 ppm as an 8-hour TWA. [29 CFR 1910.1000, published in the Federal Register 39:23542, June 27, 1974] This was apparently based upon the ACGIH TLV, except that the C designation (for Ceiling) was erroneously omitted.

Guidelines for nitric oxide, as distinct from nitrogen dioxide or "nitrous fumes", have been recommended only by the United States and the German Democratic Republic. In 1966, the American Conference of Governmental Industrial Hygienists [221] introduced a TLV of 25 ppm for nitric oxide. The recommendation by the German Democratic Republic in 1969 was 20 mg/cu m (16 ppm) as a maximum allowable concentration. [222]

Until 1954, nitric oxide levels were included in recommendations for nitrogen oxides. When the ACGIH changed the designation from nitrogen oxides to nitrogen dioxide, a limit for nitric oxide was not included. Therefore, nitric oxide technically remained without control recommendations for 12 years until 1966 when ACGIH recommended a limit of 25 ppm. [221] Nitric oxide was deemed to be about one-fifth as toxic as nitrogen dioxide based upon the animal experiments reported by Pflesser [48] in 1935 and in the review by Gray in 1959. [80] Pflesser's report [48] has been

misquoted to the effect that nitric oxide was found to be 4 or 5 times less toxic than nitrogen dioxide. [80,223] Von Oettingen [45] cited the paper correctly: "...the acute toxicity of nitrogen oxide is about four times greater than that of nitrogen dioxide but the latter is more insidious in its action..." The misinterpretation of Pflesser's paper was influential in the development of the ACGIH recommendation of 25 ppm for nitric oxide [221,223] which, in turn, is reflected in the current federal standard of 25 ppm as an 8-hour TWA (29 CFR 1910.1000) published in the Federal Register 39:23542, June 27, 1974.

Basis for the Recommended Environmental Standard

Inhalation of nitrogen dioxide in man at levels of the order of 50-100 ppm causes irritant cough, mild or transient headache, and breathlessness. [23,41,49] If the concentration of nitrogen dioxide is high enough, acute pulmonary edema [23,32,33,34,36,40,41,44,49] may develop after a characteristic delay of up to 12 hours. Just how high this concentration must be in man is not known because of the lack of environmental data from observed acute episodes. In some cases, after apparent recovery from the initial delayed pulmonary edema, without further exposure and after an interval of a few days up to 6 weeks, a second, more protracted lung condition called bronchiolitis fibrosa obliterans may develop. [34,41,44,50,51] Less commonly, bronchiolitis fibrosa obliterans may occur after an interval of several days to 6 weeks following exposure but without any initial clinical episode of acute pulmonary edema. [35,52,53] The critical concentrations and exposure times of nitrogen dioxide for these effects are not known, but there is circumstantial

evidence in most of the cited reports that the concentrations were very high, of the order of several hundred ppm. Moreover, it seems likely that most of the exposures have not been to nitrogen dioxide alone, but to mixtures of oxides of nitrogen of unknown proportions, principally nitric oxide and nitrogen dioxide.

There is some evidence that attacks of acute pulmonary edema [54] or of bronchiolitis obliterans, [51] due to severe exposures to nitrogen oxides, may be followed by chronic pulmonary impairment or insufficiency, possibly associated with peribronchiolar fibrosis [54] or centrilobular emphysema. [51]

It is not known whether prolonged low-level or repeated sporadic exposures to nitrogen oxides in the absence of attacks of acute pulmonary edema or bronchiolitis obliterans leads to emphysema in man. Kennedy [28] in 1972 attributed the extremely high prevalence (84%) of spirometric evidence of emphysema in 100 British coal miners to this cause. However, the study did not provide an adequate control group for differentiating emphysematous changes due to exposure to nitrogen oxides from other possible causes, such as coal workers' pneumoconiosis. Simple pneumoconioses, including coal workers' pneumoconiosis, are known to be associated with focal emphysema. [73]

In 1937, Vigdortschik et al [70] reported an epidemiologic study of 127 workers exposed to "oxides of nitrogen" and to "no other injurious gases" at levels "mostly below 2.8 ppm," in sulfuric acid plants and in print-etching shops. An increased prevalence of emphysema was reported in these workers, as compared with a control group matched in all respects but unexposed to "toxic substances." In addition, many other blood,

biochemical, and urinary abnormalities were reported, but the actual prevalence of the abnormalities was not given for either the exposed workers or the control group. Moreover, the assertion that the workers were exposed to nitrogen oxides alone and to "no other injurious gases" is questionable, especially with respect to workers in sulfuric acid plants. The presence of dental erosion was reported in the exposed workers, and dental erosion is known to be associated with exposure to sulfuric acid mist. [224,225,226]

In 1972, Kosmider et al [71] reported an epidemiologic study of 70 men exposed in a chemical plant to "oxides of nitrogen" 6-8 hours daily for 4-6 years. The concentrations of oxides of nitrogen expressed in terms of nitrogen dioxide fluctuated between 0.4 and 2.7 ppm. A control group of 80 male industrial workers of similar age but not occupationally exposed to oxides of nitrogen, was employed for comparison. All men smoking more than 10 cigarettes/day were excluded from both groups for the purposes of analysis. Spirometry showed slight, statistically insignificant reductions in vital capacity and maximum respiratory volume. There was a statistically insignificant degree of respiratory acidosis and metabolic alkalosis observed in the exposed men, as a group, in comparison with the controls. There was a statistically significant increase in the excretion of hydroxyproline and acid mucopolysaccharides in the urine of the exposed men, possibly indicative of connective tissue destruction. Based on the above evidence, the authors concluded that oxides of nitrogen, at the levels cited, probably cause emphysema in humans. They also found clinical evidence of chronic bronchitis (sporadic cough with mucopurulent expectoration, breathlessness on exertion, and fine moist rales in the lower lungs

on auscultation) in an unstated number of the exposed workers.

The results of these two epidemiologic studies [70,71] suggest an environmental limit for nitrogen dioxide substantially below 3 ppm, assuming that nitrogen dioxide alone is at least as toxic as "oxides of nitrogen" of unknown proportional composition at such levels. However, both studies have considerable weaknesses. The conclusion, in the first study by Vigdortschik et al, [70] that sulfuric acid plant workers were exposed to "oxides of nitrogen" and to "no other injurious gas" must be questioned, especially in view of the presence of dental erosion in some of the workers. The lack of information on how the environment was characterized, the lack of data on incidence of effects in the exposed vs unexposed groups, the doubtful significance of many of the findings, and the statistical insignificance of spirometric and blood gas changes raise doubts about the conclusions of Kosmider et al [71] in the second study.

In 1975, French [74] presented results of a retrospective study concerned with the effects of community exposure to nitrogen dioxide on acute and chronic respiratory illnesses in 3 communities located near Chattanooga, Tennessee. The data included revisions of environmental sampling measurements which were in error in the initial Chattanooga studies [75,76,77] made in 1968-69. The incidence of "acute respiratory disease" and "lower respiratory" morbidity rates was significantly higher in high-exposure (mean level of 0.083-0.219 ppm with a peak of 0.66 ppm) than in intermediate-exposure (mean of 0.06 ppm) communities as well as between intermediate- and low-exposure (mean of 0.031 ppm) communities. However, there were no significant differences among the 3 communities in the prevalence of "chronic respiratory" symptoms, such as chronic

bronchitis. Furthermore, the increase in "acute" diseases could be explained by the differences in suspended particulates observed in the three communities. It is important to note that the exposures in these communities were nearly continuous (approximately 24 hours/day) and, therefore, do not represent the type of exposure encountered in the occupational setting.

The short-term human experiments of Abe [67] reported in 1967 throw some light on acute effects of low concentrations of nitrogen dioxide. Five healthy male adults were exposed to nitrogen dioxide at 4-5 ppm for 10 minutes. Measurements of effective lung compliance, inspiratory maximum viscous resistance, and expiratory maximum viscous resistance were made prior to the gas inhalation, immediately after, and at intervals of 10, 20, and 30 minutes after inhalation had ceased. Values for effective compliance obtained 30 minutes after the cessation of exposure showed a tendency to decrease by 40% of the baseline. Expiratory and inspiratory maximum viscous resistance were unchanged immediately after completion of exposure but gradually increasing from 10 minutes after exposure, reaching a maximum at 30 minutes. Abe's results document a definite and undesirable effect at the exposure level which is the current federal standard. Moreover, the subjects were young healthy adult males, probably more fit than the average industrial worker in a population with an age range of 18-65 years.

In 1971, von Nieding and Krekeler [68] investigated the effects of low concentrations of nitrogen dioxide on the respiratory gas exchange and the airway resistance of patients with chronic bronchitis. Eighty-eight chronic bronchitis patients breathed nitrogen dioxide-air mixtures

containing 0.5-5.0 ppm for a few breaths up to 15 minutes. After inhalation of nitrogen dioxide concentrations down to 1.5 ppm the airway resistance increased significantly. Lower concentrations had no significant effect. While the end-expiratory alveolar oxygen tension remained nearly constant during inhalation of nitrogen dioxide at 4 and 5 ppm, a significant decrease of the arterial oxygen tension and, accordingly, an increase of the end-expiratory arterial tension difference for oxygen occurred. After inhalation of nitrogen dioxide at 2 ppm, there was no decrease in the arterial oxygen tension. These results indicate that exposures to nitrogen dioxide concentrations as low as 1.5 ppm may aggravate already existing respiratory impairment of sufferers from chronic bronchitis.

In 1973, von Nieding et al [69] reported further studies, including some on healthy male volunteers. The carbon monoxide diffusing capacity was measured by a single-breath method in 16 healthy male subjects before and after inhalation of nitrogen dioxide at 5 ppm for 15 minutes. A statistically significant (p less than 0.01) decrease in the diffusing capacity for carbon monoxide from 20.6 to 16.8 ml/0.1 minute/0.1 torr was observed. It is not known whether this decreased diffusing capacity would be progressive on continuation of exposure or whether it would be partially or totally reversible. Further experiments on chronic bronchitic patients indicated a significant increase in the alveolar arterial pressure following exposure for 15 minutes at 5 ppm. Increasing the exposure to 60 minutes did not change the pressure gradients from those observed after 15 minutes of inhalation.

Although a number of animal studies have been conducted in recent

years to determine the effect of exposure to nitrogen dioxide at low levels, below 5 ppm, most of the studies have employed continuous or almost continuous exposure schedules, rather than intermittent exposures parallel to the occupational exposure situation. The extrapolation of results of continuous exposure animal studies to the human occupational exposure situation has many pitfalls. As discussed earlier (see Animal Toxicity), an extrapolation from data on continuous exposure to the intermittent exposure characteristic of the occupational setting cannot be correctly performed on the assumption that effective concentration times time (Ct) is constant. In other words, the concentration producing a given effect on continuous exposure would be much lower than the concentration producing that effect by a factor greater than that predicted by differences in exposure times, and this has been verified in experimental animal exposures to soluble organic phosphate [227] as well as to nitrogen dioxide. [90,140,141]

In many of the recent animal studies, newer techniques, such as electron microscopy, biochemical and physicochemical techniques applied to lung lavage fluid, cytochemical techniques to detect cellular activity and proliferation, and bacterial and viral challenges to detect impaired resistance to infection have been introduced. Of these, some specific studies appear pertinent to man on the basis of probable similarity of pathogenesis.

In 1969, Blair et al [146] noted changes considered to be consistent with "early focal emphysema" in mice exposed at 0.5 ppm for 18 or 24 hours daily for 3-12 months. Control mice showed moderate pneumonitis but no evidence of bronchiolar obstruction or emphysema. Stephens et al [130] in

1972 reported loss of cilia, and hypertrophy and focal hyperplasia in the epithelium of the terminal bronchioles of rats exposed at 2 ppm continuously for up to 21 days. Sherwin et al [132] in 1972 reported significant increases in the average areas of alveolar walls (a change suggestive of early emphysema) in guinea pigs exposed continuously at 2 ppm for 1, 2, and 3 weeks. They also reported the replacement of Type 1 pneumocytes by Type 2 pneumocytes in the alveoli. Such a cell-type change implies thickening of the alveolar blood-gas barrier with subsequent impedance of respiratory gas exchange. In 1974, Aranyi and Port [147] demonstrated that mice exposed continuously at 2.0 ppm for 3 1/2 and 7 months or at 0.5 ppm for 1, 3, and 6 months, as well as those exposed intermittently (5 days/week) at 0.5 ppm with daily 1-hour peaks of 2 ppm (0.5/2.0 ppm) or at 0.1 ppm (0.1/1.0 ppm) with daily 3-hr peaks of 1 ppm over the same time intervals, showed no changes in blood cell counts, macrophage viability in vitro, or oxygen consumption of macrophages when compared with controls. Animals exposed at 0.5/2.0 ppm showed significant decreases of in vitro phagocytic activity of macrophages and significant morphological changes in macrophages as compared with other experimental groups and controls. The lungs of animals exposed for 7 months at 2 ppm or 0.5/2 ppm as well as those of animals exposed for 6 months to 0.1/1 ppm were said to have shown emphysematous changes in alveolar and terminal airway structures.

In contrast to the foregoing results involving continuous exposures, Wagner et al [93] in 1965 reported on intermittent exposures of 5 species of animals at 3 different levels of nitrogen dioxide for long periods. Dogs and mice were exposed at 1 and 5 ppm and rabbits, guinea pigs, rats,

and hamsters at 1, 5, and 25 ppm, all on a 6-hours/day, 5-days/week schedule, for periods ranging from 10 to 18 months. Few or no differences were noted in weight gain, blood counts, and alkaline phosphatase in dogs, or in the pathologic alterations observed in the lungs of the exposed animals of all the species studied compared with the unexposed but similarly confined control animals. The effects noted in the exposed animals may have been related to inhalation of nitrogen dioxide. However, the unexplained high incidence of similar pathologic changes in control animals may have obscured these findings. A similar problem was noted in the experiments with the tumor-susceptible mice. Mice exposed at 5 ppm for 12 months showed a higher, but statistically insignificant, incidence of tumors as compared with controls. However, the high incidence of intercurrent lesions in controls may have obfuscated experimentally induced lesions in exposed animals.

Measurement of autoimmune responses and susceptibility to challenge by pathogenic bacteria and viruses have also been used to assess the effects of exposure to nitrogen dioxide in animals. Purvis and Ehrlich [135] found a significant increase in susceptibility to infection by Klebsiella pneumoniae in mice exposed at 3.5 ppm for 2 hours. In 1970, Ehrlich et al [136] reported that mice exposed at 3.5 ppm for 2 hours and challenged with Klebsiella pneumoniae showed a significant increase in mortality relative to controls; whereas, exposures at 1.5 and 2.5 ppm, for the same period, had no effect on mortality rate. Goldstein et al [139] exposed mice at 1.9-14.8 ppm for 4 hours or 1, 2.3, and 6.6 ppm for 17 hours following infection by radiophosphorus-labeled Staphylococcus aureus. A decrease in the pulmonary bactericidal activity in the mice was observed

in the case of exposures above 7 ppm. Exposure to nitrogen dioxide at more than 2.3 ppm prior to staphylococcal challenge also caused decreased bactericidal activity.

Coffin et al [140] studied the time-dose relationship between intermittent and continuous exposures to nitrogen dioxide and mortality in mice challenged with Streptococcus pyogenes (Group C). Results of single equivalent Ct (concentration x time) exposures indicated that concentration was more important in determining the rate of mortality than time. Exposures at 2.3 ppm for 3 hours and 1 ppm for 7 hours did not appear to increase mortality above that observed in control animals. Continuous exposure at concentrations of 0.5 and at 3.5 ppm, as well as intermittent exposure (7 hours/day) at 3.5 ppm, resulted in a significant increase in mortality. Insufficient data were collected on continuous exposures at 1.5 ppm to determine the significance of the relationship between mortality and total time of exposure, or the significance of differences in mortality between this group and animals exposed at 0.5 or 3.5 ppm. Intermittent exposure at 3.5 ppm resulted in a lower mortality rate than continuous exposure at the same concentration.

Studies by Ehrlich [155] and Ehrlich and Henry [156] indicated increased susceptibility or mortality in mice challenged with airborne Klebsiella pneumoniae and exposed at 0.5 ppm continuously for 3 months or intermittently (6-18 hours/day) for 1 year. More recently, Ehrlich et al [158] vaccinated Swiss albino mice with A2/Taiwan influenza virus vaccine following nearly continuous exposure to nitrogen dioxide at 2 ppm, to nitrogen dioxide at 0.5 ppm with daily 1-hour peaks of 2 ppm (0.5/2.0 ppm), or to filtered air. Exposures were continued for 28 weeks following

vaccination, and animals were killed at intervals of 2, 4, 8, 12, 16, 20, 24, and 28 weeks. None of these exposures had a significant effect on HI antibody titers. Serum neutralizing (SN) titers were significantly below controls two weeks after vaccination in animals exposed at 0.5/2.0 ppm, but not in those exposed continuously at 2.0 ppm. In general, exposure at 0.5/2 ppm had as much of an effect, if not a greater effect, on serum IgA, IgM, IgG1, and IgG2 levels as exposure at 2.0 ppm. Whether or not there is a direct relationship between the levels of immunoglobulins and chronic pulmonary disease is still a matter of speculation. [158]

No epidemiologic studies have been found which would indicate a carcinogenic or other mutagenic effect from human exposure to nitrogen dioxide. Results from animal exposures are inconclusive. Adenomatous changes have been noted in the lungs of mice intermittently exposed at 40 ppm for 18 months [160] and hamsters continuously exposed at 40 ppm nitrogen dioxide and 20 ppm nitric oxide for 16 months. [161] But, carcinomas were not observed in the experimental animals. Other studies [93,124,163] suggest a tumor provoking or a cocarcinogenic effect of exposure to nitrogen dioxide. However, the findings of these studies are either limited in scope or of such questionable significance that it is not possible to attribute such actions to inhalation of nitrogen dioxide from the presently available evidence. Reports of human mutagenesis resulting from exposure to nitrogen dioxide have not been found in the literature.

Epidemiologic and animal studies which clearly delineate a safe level for human exposure to nitrogen dioxide are not yet available. Existing epidemiologic studies contain, for the most part, errors, omissions, and inconsistencies and are, therefore, unreliable for establishing a safe

exposure level. Difficulties in interpreting the animal data reviewed below may be attributed to a number of variables, such as species-specific responses to exposure, criteria of effect, and type (continuous versus intermittent) and duration of exposure.

Tyler et al [103] noted a number of important anatomic differences between species with respect to the vasculature of the terminal airways and spaces. These anatomic differences, coupled with differences in metabolism and other physiologic characteristics (eg, respiration rate) of various animal species, may differentially affect the deposition of inhaled nitrogen dioxide in lung tissue, and thereby give rise to species-specific pulmonary responses to exposure. Such species specificity has been indicated either by the time of onset of microscopic pathologic changes or by the toxicologic manifestations of different animal species exposed to nitrogen dioxide under the same experimental conditions. [90,93] Therefore, extreme caution must be taken in attempting to directly extrapolate experimental animal data to the responses of humans exposed to nitrogen dioxide. Considerably more effort must be expended to determine appropriate animal models of human pulmonary function so that present and future experimental animal data may be used in defining safe limits of occupational exposure.

Several criteria have been used to evaluate the toxic effects of exposure in animals. These criteria include abnormal changes in respiration, cellular morphology of the pulmonary system, weight, reproduction, and immunoglobulin levels. Susceptibility to viruses and bacteria, as well as hematologic and biochemical changes, have also been used to assess nitrogen dioxide inhalation toxicity. Of these criteria,

changes in immunoglobulin levels and susceptibility to viral and bacteria infection are least relevant to defining safe exposure levels in humans. Although elevated levels of serum immunoglobulins have been correlated with chronic lung disease, cause-and-effect relationships have not been documented. [158] Furthermore, evidence of an increased incidence of bacterial and viral infections in workers exposed to nitrogen dioxide has not been found. Indeed, a well-designed and controlled field study is needed to confirm such effects in humans.

With respect to the remaining criteria, effects noted are highly dependent upon the animal species, exposure schedule, and total time of exposure. Thus, rats continuously exposed at 0.8 ppm over their natural lifetime showed respiration rates 20% above controls. [144] However, beagle dogs exposed to nitrogen dioxide at 0.5-1.0 ppm plus nitric oxide at 0.2 ppm oxide for 16 hours/day over 18 months did not differ significantly from controls in single-breath carbon monoxide diffusing capacity, dynamic pulmonary compliance, and total pulmonary resistance. [148] Steadman et al [145] found that monkeys, dogs, rabbits, guinea pigs, and rats continuously exposed for 90 days at 0.5 ppm had a slight weight loss. In contrast, Wagner et al [93] reported no significant difference in body weight gain between control animals and animals exposed intermittently 6 hours/day, 5 days/week for up to 18 months. In terms of reproduction, Shalamberidze and Tsereteli [142] observed a prolongation of the estrus cycle and a reduction in fetal weights in female albino rats exposed at 1.3 ppm 12 hours/day for 3 months. Such findings were not observed in rats exposed at 0.07 ppm under the same experimental conditions. Apparently, the threshold for reproductive alterations in albino rats lies somewhere between 0.07 and

1.3 ppm; however, the specific concentration at which these changes begin to occur is unknown.

Minimum concentrations of exposure to nitrogen dioxide leading to macro- and microscopic changes associated with chronic obstructive disease of the lungs have not been determined. Early bronchiolar inflammation, expansion of lung alveoli, and alveolar lesions reportedly consistent with focal emphysema have been observed in mice exposed at 0.5 ppm for 6, 18, or 24 hours/day for 3-12 months, [146] and at 0.5 ppm with daily 1-hour peaks at 2 ppm for 5 days/week for 3 1/2 to 7 months. [147] Similar changes have been observed in the lung tissue of guinea pigs exposed at 1 ppm, 8 hours/day for 180 days. Conversely, only insignificant macro- and microscopic changes in lung tissue have been observed in dogs, rabbits, guinea pigs, rats, and hamsters exposed at 1 ppm, 6 hours/day for up to 18 months, [93] and in rats exposed continuously for 16 weeks at concentrations of 0.8-4.0 ppm. [143]

Although it is difficult to extrapolate animal data to occupational exposure and to human response to nitrogen dioxide inhalation, a number of important principles have emerged from animal research. First, it is apparent that a specific concentration of nitrogen dioxide on intermittent exposure is considerably less toxic than on continuous exposure. [93,140] Second, the toxic hazard associated with nitrogen dioxide during continuous exposure is primarily determined by the peak and not by the average concentration of exposure. The latter is supported by data which indicate equivalent or nearly equivalent effect on the severity of experimental respiratory infections from continuous exposure at 2.0 ppm and from continuous exposure at 0.5 ppm with 1-hour peaks at 2.0 ppm, [147,158] as

well as by evidence indicating that for equal Ct's, brief high level exposures are more hazardous than longer exposures at low concentrations. [90,140,141]

The current federal standard for nitrogen dioxide of 5 ppm was adopted from the ACGIH recommended Threshold Limit Value, except that the C designation (for ceiling) was erroneously omitted. According to the current documentation, [219] a 5-ppm ceiling should "insure against immediate injury or adverse physiological effects from prolonged daily exposure." However, evidence obtained since the time of this documentation suggests that humans with normal respiratory function may be acutely affected by exposure at or below this level. Furthermore, the conditions of workers with chronic respiratory diseases, such as chronic bronchitis, may be aggravated by exposure to nitrogen dioxide at a concentration of approximately one-third of the current federal standard. [68,69] Although much of the animal data are inconsistent and, thereby, inconclusive at this time, some studies have indicated chronic effects on respiration, [129,144] cellular morphology of the pulmonary system, [71,99,129,132-134, 146,147,154,157] reproduction, [142] immune responses, [116,117,155, 156,158] and weight gain [127] in animals exposed to nitrogen dioxide at and below the current federal standard. In view of these results, it is concluded that the federal standard of 5 ppm should be reduced.

A reduction in effective lung compliance with a corresponding increase in inspiratory and expiratory maximum viscous resistance as well as significant decreases in arterial oxygen tension and single-breath carbon monoxide diffusing capacity have been noted in normal adult males exposed to nitrogen dioxide for 10-15 minutes at 4-5 ppm. [67-69] The

threshold for the aforementioned changes in normal human respiratory mechanics is unknown, but it is obviously below 4 ppm.

Data indicating respiratory effects and toxicologic changes of the pulmonary system in animals exposed to nitrogen dioxide for either brief or long durations appear to be inconsistent or inconclusive below 2 ppm. However, studies [68,69] conducted on persons with chronic respiratory disease (bronchitis) indicate that 15-minute exposure to nitrogen dioxide at concentrations above 1.5 ppm but not at or below this level results in a decrease in arterial oxygen partial pressure, and in increases in alveolo-arterial pressure gradients and airway resistance, all of which may aggravate existing respiratory problems. Similar changes in arterial oxygen partial pressure, alveolo-arterial pressure gradients, and airway resistance have been observed in healthy subjects exposed at 4-5 ppm. Although the specific concentration at which these changes begin to occur in normal human subjects is unknown, it is likely to be at about the same or perhaps a slightly higher concentration than the one inducing pulmonary changes in humans with existing chronic bronchitis. Therefore, the environmental limit should be reduced to a ceiling value of 1 ppm to prevent acute irritant effects in the lungs of workers exposed to nitrogen dioxide. In addition, the prevention of repeated acute episodes of irritancy should lessen the risk of developing chronic obstructive lung disease.

Concerning nitric oxide, there is no direct quantitative basis for an environmental limit. No environmental data are available on exposures to nitric oxide alone. Furthermore, it is improbable that exposures to nitric oxide alone, as opposed to a mixture of nitric oxide and nitrogen dioxide,

would occur in an occupational situation. Even under experimental circumstances, it would be difficult to achieve human exposures to nitric oxide in the absence of higher oxides of nitrogen, particularly nitrogen dioxide, at concentrations above about 100 ppm because of the rapid rate of oxidation to nitrogen dioxide. [167] At concentrations below 100 ppm, it might be feasible to achieve experimental exposures to nitric oxide virtually free of nitrogen dioxide because of the much slower rate of oxidation, [10] but such experiments addressed to toxicity have not been reported. One experiment on seven human volunteers exposed at 5, 1, 0.5, and 0.33 ppm by inhalation through the mouth has been reported. [62] However, this study presented only the proportion of the inhaled gas which was absorbed, and no subjective or objective effects or durations of exposure were mentioned. The proportionate absorption of the inhaled gas containing nitric oxide or nitrogen dioxide was found to be virtually the same.

In 1941, McCord et al [56] reported a study of 4 arc-welders who were exposed, for an unstated period of time, to "nitrous gas" expressed as nitrogen dioxide at levels ranging from 2.0 to 10.3 ppm. Independent experiments have demonstrated [5] that, in the presence of an electric arc, the initial proportion of nitric oxide present in the "nitrous gas" may be above 90%. At nitric oxide concentrations around 10 ppm, the rate of oxidation is very slow (by calculation, it would take 2-3 hours for a 25% conversion to nitrogen dioxide at 20 C). [167] Therefore, it is inferred that the four welders observed by McCord et al [56] were exposed predominantly to nitric oxide rather than to nitrogen dioxide. The welders all had methemoglobin present in their blood to the extent of 2.3, 2.3,

2.5, and 2.6%, respectively. A more recent British study by Morley and Silk [63] in 1970 reported on a larger number (31) of welders and oxyacetylene flame-cutters. The workers were exposed to oxides of nitrogen, measured as nitrogen dioxide, at levels ranging up to 115 ppm. In no case was an increase of methemoglobin detected by a spectrophotometric methed.

The British anesthetic gas accidents reported in 1967 [58] would initially seem to present 2 cases of nitric oxide poisoning, inasmuch as this gas contaminated a cylinder of anesthetic nitrous oxide. As long as the nitric oxide remained mixed with nitrous oxide alone, it would remain as nitric oxide. [167] However, the nitric oxide-contaminated nitrous oxide was mixed first with 25% then 50% oxygen in the anesthetic apparatus. As the initial concentration of nitric oxide in the cylinder was stated to be "in excess of 1.5%," it would theoretically have been oxidized to nitrogen dioxide in less than 8 seconds. [167] Therefore, the exposure of the two patients might have been effectively to a mixture of nitric oxide and nitrogen dioxide, both gases being present in highly toxic concentrations and together probably accounting for the effects observed. Both patients became deeply cyanotic within 3 minutes or less of inhaling the gas mixture. The presence of an unspecified amount of methemoglobin was reported in the first patient's blood shortly after the exposure, but it was absent after 4 hours. The patient showed signs of great respiratory distress and died in cardiac arrest 18 1/2 hours after the commencement of the anesthetic. At autopsy, pulmonary edema was confirmed. When the second patient became severely cyanotic from the same anesthetic mixture, it was surmised that something was wrong with the anesthetic, its

administration was discontinued, and 100% oxygen was given. The patient showed signs of some respiratory distress but made a complete recovery. It must be stressed that the levels of exposure to nitric oxide and nitrogen dioxide suggested in these two cases are speculated approximations only. The actual gas mixture which was administered to the two patients was never analyzed; however, contamination of the nitrous oxide with nitric oxide in excess of 1.5% was determined from analyses performed by the manufacturer on other cylinders from the same production batch. [58] In addition, if fractional distillation had occurred, [167] the first gas released from the cylinder would probably have been primarily nitric oxide; thus, the first patient might have been exposed to nitric oxide in excess of 1.5%.

From these two patient exposures to nitric oxide, probably mixed to a varying degree with nitrogen dioxide, it is apparent that high concentrations (in the thousands of ppm) rapidly cause cyanosis, methemoglobinemia, and possibly death. [58] Severe lung irritation and pulmonary edema may be attributed to possible nitrogen dioxide exposure. Observations and data at lower levels of exposure to nitric oxide alone are unclear concerning a minimal level for toxic effects in humans.

Reported animal studies also provide extremely limited information on nitric oxide. In the 1930's, Pflesser [47,48] stated that the lethal concentration for 100% (LC100) of an unspecified number of white mice was about 350 ppm, the LC50 was 320 ppm, and that at 310 ppm all the animals survived an 8-hour exposure--a rather steep dose-response curve. [47,48] A subsequent study reported in 1962 by Paribok and Grokholskaya, [87] employing essentially the same experimental technique in mice as used by Pflesser, found that it took 6 hours of exposure to nitric oxide at 322 ppm

to produce a methemoglobinemia of 60%. Guinea pigs were also exposed for an unstated period to nitric oxide at 175 ppm. Little effect was observed on the rate of recovery to resting respiratory rhythm after treadmill exercise. The manner in which the mortality data on mice were presented prevent comparison with Pflesser's LC50 findings.

Von Oettingen's statement [45] that no cases of nitrogen oxide (nitric oxide) poisoning in humans had been reported in the literature is essentially true, considering the intimate association which exists between nitric oxide and nitrogen dioxide. Human [58] and animal [48,87,88] exposures to nitric oxide, whether relatively pure or as mixed with other nitrogen oxides, indicated it to be nonirritant to the respiratory tract and that it produced methemoglobinemia and rapid cyanosis at high concentrations (approximately 1,000 ppm and higher). At these levels, nitric oxide was more toxic than nitrogen dioxide. Nitrogen dioxide, on the other hand, produced pulmonary irritation followed by edema at lower concentrations. Nitric oxide, on the basis of lethality observed in animals following 2 to 8-hour exposures, had been considered to be less toxic than nitrogen dioxide [80] by a factor of 4-5 times.

At lower and sublethal concentrations (eg, 175 ppm) and 8-hour exposures, nitric oxide was found to be less toxic than nitrogen dioxide as indicated by changes in oxygen consumption and postexercise respiratory recovery. [87]

At the present time, there are no definitive data in the scientific literature concerned with chronic effects in humans or animals exposed to nitric oxide at low concentrations. It is known [46,48,58,86] that exposures at high concentrations of nitric oxide result in

methemoglobinemia and cyanosis in both humans and experimental animals, so an environmental limit is obviously needed. In the absence of data showing toxic effects for humans and animals exposed at and below 25 ppm, it is believed that the current federal standard of 25 ppm should be continued as a TWA for up to 10 hours/day and 40 hours/week to protect workers from exposure to nitric oxide. Future research should be conducted to determine concentrations of nitric oxide that result in impairment of respiratory or other biologic functions so that a soundly based standard may be promulgated.

It is recognized that many workers are exposed to the oxides of nitrogen at ambient air concentrations or at concentrations considerably below the recommended occupational limits. Under these conditions, it should not be necessary to comply with many of the provisions of this recommended standard. However, concern for worker health requires that protective measures be instituted below the enforceable limits to ensure that exposures do not exceed the standard. For this reason, "occupational exposure to the oxides of nitrogen" has been defined as exposure above half the environmental limits, thereby delineating those work situations which do not require the installation of unnecessary controls and the expenditure of health resources for provisions such as environmental and medical monitoring, and associated recordkeeping.

VI. COMPATIBILITY WITH AMBIENT AIR QUALITY STANDARDS

National primary and secondary air quality standards for nitrogen dioxide were published by the Environmental Protection Agency in the Federal Register 30:8186-8201, April 30, 1971 (present codification in 40 CFR 50.1-50.3, 50.11) The national primary air quality standards define levels of air quality which are judged necessary, with an adequate margin of safety, to protect the public health. The national secondary air quality standards define levels of air quality which are judged necessary to protect the public welfare from any known or anticipated effects of a pollutant. The term "ambient air", as used in the air quality standards, means that portion of the atmosphere, external to buildings, to which the general public has access.

The national primary and secondary ambient air quality standards for nitrogen dioxide are 100 μg/cu m (0.05 ppm) annual arithmetic mean, measured by a specified reference method. However, on June 14, 1972 (Federal Register 37:11826, June 14, 1972), it was announced that the reference method would be reevaluated because of apparent deficiencies. On June 8, 1973, tentative candidate methods from which to select a replacement reference method were published. (40 CFR Part 50)

No direct comparison can be made between the national primary and secondary ambient air quality standards and the recommended standard for occupational exposure because the levels of exposure to the general public involve varying health status and age on a 24-hours/day, 7-days/week basis. The ambient air quality standards should be substantially lower than the occupational standards which are based on a workday of up to 10 hours and a workweek of 40 hours.

VII. RESEARCH NEEDS

Epidemiologic Studies

Three epidemologic studies (56,70,71) indicated that chronic effects on the pulmonary system as well as hematologic changes may be observed in workers exposed to oxides of nitrogen at and below the current federal limits. However, each of these studies contained errors or omissions in at least one of the following categories: (1) inadequate characterization of exposure concentration or duration of exposure, (2) exposure to gases in addition to the oxides of nitrogen which may induce similar toxic effects, and (3) inconsistent or conflicting results on the toxic effects observed. Therefore, carefully controlled cross-sectional studies which adequately characterize the environment and control for exposures to airborne contaminants other than the oxides of nitrogen should be conducted.

Workers exposed to oxides of nitrogen should be compared with normative data obtained from appropriate control subjects on measures of the pulmonary system including ventilatory mechanics and spirometry as well as hematologic indices, such as the percentage by volume of methemoglobin. Attempts should also be made to determine the risk of acute and chronic obstructive pulmonary disease as a function of concentration and time of exposure to oxides of nitrogen.

Chronic Studies in Animals

Although a number of animal studies indicated chronic changes in respiration, [129,144] cellular morphology of the pulmonary system, [71,99,129,132,133,134,146,147,154,157] reproduction, [142] immune

responses, [116,117,155,156,158] and weight gain [127] resulting from long-term exposure to oxides of nitrogen, most of these studies used continuous exposures which are atypical of the occupational setting. Furthermore, some studies have indicated a regression or a reversal of toxic effects either during exposure [95,130,131] or during the postexposure recovery period. [91,92,94,95] Such data suggest the possibility of adaptation to inhalation of oxides of nitrogen or regeneration and repair of pulmonary tissues during times in which the subject is away from the exposure environment. Therefore, future studies investigating the chronic effects of long-term exposure to oxides of nitrogen in animals should utilize exposure schedules similar to those observed in industry, ie, 6-10 hours/day, 5 days/week. Dose-effect relationships should be generated on dependent variables, such as respiration rate, pulmonary mechanics, morphologic changes of pulmonary tissue, incidence of chronic obstructive diseases of the pulmonary system, methemoglobin levels, and autoimmune responses at concentrations corresponding to 1/10-2 times the recommended limits. Particular emphasis should be placed on collecting data on exposures to nitric oxide since the data base concerned with the toxicity of this oxide of nitrogen is minimal at this time.

Reproduction

Shalamberidze and Tsereteli [142] observed significant changes in estrus, litter size, and fetal weights in rats exposed to nitrogen dioxide at 1.3 ppm for 12 hours/day over a 3-month period. No effects were noted in animals exposed for the same time at 0.07 ppm. Further research is needed to define the limits of exposure at which these changes take place

in the rat and in other animal species. If it can be demonstrated that low-level exposure to oxides of nitrogen consistently results in changes in the reproductive system of various animal species, then special restrictions on exposure of female workers to oxides of nitrogen must be implemented.

Mutagenicity

Nitrous acid has been shown to have a potent mutagenic effect on lower forms of life, such as tobacco mosaic virus [165] and Escherichia coli. [166] Although nitric oxide and nitrogen dioxide may combine with water to form nitrous acid under certain conditions, evidence of mutagenesis by exposure to these oxides of nitrogen per se have not been found in the literature. Therefore, studies of the possible mutagenicity of nitric oxide and nitrogen dioxide, including microbial screens, should be conducted.

Carcinogenicity

The results of three studies [160,161,162] reviewed in this document suggest that either continuous or intermittent exposure to oxides of nitrogen at fairly high concentrations does not result in a significant increase in neoplastic changes in mice, hamsters, and rats. NIOSH has been informed of a number of studies which are currently in progress, the results of which are yet incomplete or inconclusive. Thus, although available evidence suggests no direct cause-and-effect relationship between inhalation of oxides of nitrogen and the development of neoplasms, NIOSH believes that results of on-going research and future research in this area

must be obtained before a final conclusion is made.

Of particular concern is the possible effect of oxides of nitrogen in combination with other occupational contaminants, such as hydrocarbons, fibrous dusts, and organic solvents. Preliminary evidence [163] suggests a possible synergism between nitrogen dioxide and benzo(a)pyrene in producing squamous cell carcinomas in rats where the concentration of nitrogen dioxide used in this study was 25 times greater than the recommended ceiling limit. Recently, there has been an increasing concern about the in vivo or airborne formation of N-nitroso compounds from oxides of nitrogen and primary or secondary amines. The results of several animal studies indicate that inhalation of some secondary nitrosamines at high concentrations can result in acute liver damage and severe hematologic changes, [182] whereas long-term exposure at lower concentrations produces carcinomas in the trachea and lungs. [184,185] Although secondary nitrosamines have been shown to form from precursors in the gastrointestinal tract, [178] similar data have not been found on in vivo formation in the pulmonary system. Furthermore, no animal data or epidemiologic data have been found which can link cancer to nitrosamine exposure at concentrations observed in occupational environments. It is evident that research must be initiated to answer these important questions concerned with exposure to nitrosamines and that a concentrated effort must be directed toward determining the possible additive, synergistic, or inhibitory effects of oxides of nitrogen in combination with hydrocarbons, fibrous dusts, and organic solvents on neoplastic dose-response relationships.

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IX. APPENDIX I

METHOD FOR SAMPLING NITROGEN DIOXIDE AND NITRIC OXIDE

The recommended method is adapted from procedures described by Blacker [211] and by Levaggi et al [212]. This method is highly adaptable to personal monitoring, and it offers a number of advantages over available bubbler-type methods.

Principle

The following sampling method for nitrogen dioxide and nitric oxide in air employs collection of the NO2 on a triethanolamine-impregnated molecular sieve surface, oxidation of the NO to NO2 by a solid oxidizer, and collection of the converted NO on another section of triethanolamine-impregnated solid sorbent. The trapped NO/NO2 is removed with an absorbing solution and the concentrations are determined by reading the color of the solution with a spectrophotometer.

General Requirements

Both nitrogen dioxide and nitric oxide concentrations shall be determined within the worker's breathing zone and shall meet the following criteria in order to evaluate conformance with the standard:

(a) Samples collected shall be representative of the individual worker's exposure.

(b) Sampling Data Sheets shall include:

(1) The date and time of sample collection.

(2) Sampling duration.

(3) Volumetric flow rate of sampling.

(4) A description of the sampling location.

(5) Other pertinent information.

Breathing Zone Sampling

Breathing-zone samples shall be collected as near as practicable to the worker's face without interfering with his or her freedom of movement and shall characterize the exposure from each job or specific operation in each production area.

(a) Sampling Equipment

A calibrated personal sampling pump capable of operating at 50 cc/min, ±5%, and a solid sorbent sampling tube containing 400 mg of triethanolamine (TEA)-impregnated molecular sieve (type 13X, 30-40 mesh molecular sieve), a gap of 12 millimeters, 800 mg of #1900277 oxidation material from the Dragerwerk Company of Germany, and another 400 mg section of the TEA sorbent, all packed in a 5-mm ID, 7-mm OD glass tube. The ends of the tube are flame sealed (see Figure XIII-1).

(b) Reagents

(1) Purity -- All chemicals should be analytical grade.

(2) Nitrogen dioxide absorbent -- Add 25 g of triethanolamine to a 250-ml beaker; add 4 g of glycerol, 50 ml of acetone, and sufficient distilled water to bring the volume up to 100 ml. To the mixture add about 50 cc of 30- to 40-mesh molecular sieve 13X (MCB No. MX 1583-1). Stir and let stand in the covered beaker for about 30 minutes. Decant the excess liquid and transfer the molecular sieve to a porcelain pan which is then placed under a heating lamp until most of the moisture has evaporated. Complete the drying in an oven at 110 C for 1 hour. Store in a closed glass container. [211]

(3) Oxidizer -- The oxidizer used in the sampling train is from Dragerwerk of The Federal Republic of Germany. Exact preparation of this oxidizer is proprietary, but the material is available from Dragerwerk through its US distributor, National Mine Safety Company. Substances of equivalent oxidizing characteristics may be used.

(c) Sampling Procedure

Immediately before sampling, the ends of the sampling tube are broken to provide an opening approximately one-half the internal diameter of the tube. The tube should be placed in a vertical position during sampling to avoid channeling. Air being sampled should not be passed through any hose or tubing before entering the sampling tube. Set the flow rate at 50 cc/min and record the starting time and initial counter reading (if applicable). The sampling tube is attached to the worker's clothing to sample from the worker's breathing zone. The sample is collected at 50 cc/min for a minimum 15-minute period. A minimum sample volume of 0.75 liter must be taken to measure 0.5 ppm NO2. Sample volumes should not exceed 1.5 liter if nitric oxide concentrations of 50 ppm or greater are expected. When the sample has been taken, record the time and final counter reading.

The tubes should be sealed with appropriate plastic caps immediately after sampling. Masking tape is a suitable substitute for sealing the tubes. One sampling tube should be handled in the same manner as the sample tubes (break, seal, and transport), except for the taking of an air sample. This tube should be labeled as a blank. At least one blank tube should be provided for every 20 samples.

Shipping

After sampling, the tubes should be capped and labeled. The tubes should be packed with cushioning material to prevent the tubes from being broken in transit.

Calibration of Sampling Trains

Since the accuracy of an analysis can be no greater than the accuracy of the volume of air which is measured, the accurate calibration of a sampling pump is essential to the correct interpretation of the pump's indication. The frequency of calibration is dependent on the use, care, and handling to which the pump is subjected. In addition, pumps should be recalibrated if they have been misused or if they have just been repaired or received from a manufacturer. If the pump receives hard usage, more frequent calibration may be necessary.

Ordinarily, pumps should be calibrated in the laboratory both before they are used in the field and after they have been used to collect a large number of field samples. The accuracy of calibration is dependent on the type of instrument used as a reference. The choice of a calibration instrument will depend largely upon where the calibration is to be performed. For laboratory testing, primary standards such as a spirometer or soapbubble meter are recommended, although other standard calibrating instruments such as a wet test meter or dry gas meter can be used. The actual setup will be the same for all instruments. Instructions for calibration with the soapbubble meter follow. If another calibration device is selected, equivalent procedures should be used.

(a) Flowmeter Calibration Test Method

For calibration of low flow pumps, ie, 50-200 cc/min, a soapbubble meter with calibration marks from 1 to 100 ml is recommended.

(1) Procedure

(A) Check the voltage of the pump battery with a voltmeter to assure adequate voltage for calibration. Charge the battery if necessary.

(B) Assemble the sampling train as shown in Figure XIII-2, connecting the sampling tube in line between the soapbubble meter and the water manometer and then connecting the personal sampling pump after the manometer.

(C) Turn the pump on and moisten the inside of the soapbubble meter by immersing the buret in the soap solution and draw bubbles up the inside until they are able to travel the entire buret length without bursting.

(D) Adjust the pump rotameter to provide a flow rate of 50 cc/minute.

(E) Check the water manometer to ensure that the pressure drop across the sampling train does not exceed 2.5 inches of water.

(F) Start a soapbubble up the buret and, with a stopwatch, measure the time it takes for the bubble to move from one calibration mark to another. For a 100-ml buret, a convenient calibration volume is 50 ml. At the same time, record the initial and final counter readings as the bubble travels between the chosen calibration marks on the bubblemeter. This can be accomplished by quickly reading the counter

numbers when the soapbubble passes the calibration marks while the pump is running, or the pump can be turned off when the soapbubble reaches the final calibration mark and a counter reading taken.

(G) Repeat the procedure in (F) above at least 2 times, average the results, and calculate the flow rate by dividing the volume between the preselected marks by the time required for the soapbubble to traverse the distance. Also calculate the pump stroke factor (cc/stroke) by subtracting the initial counter reading from the final counter reading, thus obtaining the number of strokes, and dividing this number into the volume over which the strokes were recorded.

(H) Data for the calibration include the volume measured, number of pump strokes measured, elapsed time, pressure drop, air temperature, atmospheric pressure, serial number of the pump, date, and name of the person performing the calibration.

X. APPENDIX II

ANALYTICAL METHOD FOR NITROGEN DIOXIDE AND NITRIC OXIDE

The recommended method is based on procedures described by Blacker [211] and by Levaggi et al. [212]

Principle

The nitrogen dioxide absorbed on the first section of molecular sieve and the nitrogen dioxide, after oxidation of nitric oxide to nitrogen dioxide, on the second section of molecular sieve are treated with sulfanilamide and N-1-naphthylethylenediamine dihydrochloride. Absorbance of the color developed is measured at 540 nm.

Apparatus

Spectrophotometer -- A laboratory instrument suitable for measuring the absorbance at 540 nm, utilizing 1-cm cells or larger.

Reagents

Absorbing solution -- Dissolve 15.0 g of triethanolamine in approximately 500 ml of distilled water, add 0.5 ml of n-butanol, and dilute to 1 liter.

Hydrogen peroxide -- Dilute 0.2 ml of 30% hydrogen peroxide to 250 ml with distilled water.

Sulfanilamide -- Dissolve 10.0 g of sulfanilamide in 400 ml of distilled water. Add 25 ml of concentrated phosphoric acid, mix well, and dilute to 500 ml.

NEDA -- Dissolve 0.5 g of N-1-naphthylethylenediamine dihydrochloride in 500 ml of distilled water. This solution will be stable for several

months if kept well-stoppered in a brown bottle in the refrigerator. Alternatively, weighed small amounts of the solid reagent may be stored.

Standard solution -- Dissolve 150.0 mg of reagent-grade sodium nitrite in distilled water, and dilute to 1 liter. This solution contains 100 μg of NO2 (ion)/ml.

Preparation of Standard Curve

Dilute 2 ml of stock standard (100 μg of NO2 (ion)/ml) to 100 ml with absorbing solution to prepare a solution containing 2 μg of NO2 (ion)/ml. To a series of 25-ml glass-stoppered graduated cylinders add 0, 1, 3, 5, and 7 ml of standard (2 μg of NO2 (ion)/ml). Add absorbing solution to bring the volume in each cylinder up to 10 ml. Add 1 ml of hydrogen peroxide solution, 10 ml of sulfanilamide solution, and 1.4 ml of NEDA solution, with thorough mixing after the addition of each reagent. Allow 10 minutes for complete color development and measure absorption at 540 nm, using the treated blank to zero the spectrophotometer. Prepare the standard curve by plotting absorbance versus concentration.

Procedure

With a tweezer remove and discard the glass wool plug from one end of the nitrogen dioxide sample tube (Appendix I) and transfer the molecular sieve to a 25-ml glass-stoppered graduate. Add absorbing solution to make the volume up to 20 ml, and then shake vigorously for about 30 seconds. Allow a few minutes for the solids to settle, and then transfer 10 ml to a 25-ml glass-stoppered graduate. Prepare a blank in the same manner from unexposed molecular sieve obtained from an unused nitrogen dioxide absorber tube. To the sample and the blank add 1 ml of hydrogen peroxide solution,

10 ml of sulfanilamide solution, and 1.4 ml of NEDA solution with thorough mixing after the addition of each reagent. Allow 10 minutes for complete color development and measure absorbance at 540 nm, using the treated blank to zero the spectrophotometer. Determine NO2 (ion) in the aliquot of the sample from the standard curve.

Calculations

$$\text{ppm (v/v) of NO2 (gas)} = \frac{\mu\text{l NO2 (gas)}}{\text{liters (air)}}$$

1 μl NO2 (gas) = 1.88 μg NO2 (gas) at 25 C and 760 mmHg

1 μg NO2 (gas) = 0.63 μg NO2 (ion)

If

r = sampling rate, liters of air/minute, and

t = sampling time in minutes,

$$\mu\text{l NO2 (gas)} = \frac{\mu\text{g NO2 (ion) in aliquot x 2}}{1.88 \times 0.63}$$

$$= \mu\text{g NO2 (ion) in aliquot x 1.69, then}$$

$$\text{ppm NO2 (gas)} = \frac{\mu\text{g NO2 (ion) in aliquot x 1.69}}{\text{r x t}}$$

The concentration of nitric oxide, expressed in ppm of nitrogen dioxide (gas), is equivalent to the concentration of nitrogen dioxide as calculated above.

XI. APPENDIX III

DETERMINATION OF EXPOSURE AREAS TO NITROGEN DIOXIDE WITH DETECTOR TUBES AND WITH PORTABLE DIRECT-READING INSTRUMENTS

Detector Tubes

(a) Atmospheric Sampling

(1) Equipment Used

A typical sampling train consists of a detector tube with a corresponding sampling pump. A specific manufacturer's pump may only be used with his detector tubes because these are normally integrated units.

(2) Sampling Procedures

A specific procedure depends on the manufacturer's instructions but normally consists of breaking both tips off a detector tube, inserting the tube into the pump, and taking a specific number of strokes with the pump.

(3) Handling and Shipping of Samples

Detector tubes are not stable with time because the stain in some tubes fades in a few minutes. The tubes should be read immediately in accordance with the manufacturer's instructions and charts and no attempt should be made to save the used tubes.

(b) General Principles

Gas detector tubes contain a chemically impregnated packing which indicates the concentration of a contaminant in the air by means of a chemically produced color change. The color changes are not permanent or stable, so the stained tubes must be read immediately after the samples are

taken. The length of stain or color intensity is read according to the manufacturer's instructions and may involve comparing the stain with a chart, a color comparator, or a direct concentration reading from calibration marks on the tube. Detailed descriptions are provided by individual manufacturer's instructions.

Tubes obtained from commercial sources which bear the certified seal of NIOSH adhere to the requirements as specified for Certification of Gas Detector Tube Units in 42 CFR Part 84 (Federal Register 38:11458, May 8, 1973). A user may perform his own calibration on commercially acquired tubes by generating accurately known concentrations of nitrogen dioxide in air and correlating concentration with stain length or color intensity.

The use of detector tubes with their respective pumps for compliance purposes is inappropriate because sampling times are necessarily very brief; thus, an excessive number of sampling periods would be required to permit calculation of a time-weighted average in the case of nitric oxide. In addition, the accuracy of detector tubes is limited [see (e) below].

(c) Range and Sensitivity

Certification standards require that certified tubes have a range of from 1/2 to 5 times the time-weighted average concentration for the duration of the sampling period. The sensitivity varies with tube brands.

(d) Interferences

Interferences vary with tube brands. The manufacturer's instructions must be consulted.

(e) Accuracy

Certification standards under the provisions of 42 CFR Part 84 (Federal Register 38:11458, May 8, 1973) specify reliability to within ±25%

of the actual concentration in the range of 0.75 to 5 times the standard and ±35% in the range from 0.5 up to, but not including, 0.75 times the standard.

(f) Advantages and Disadvantages

The use of detector tubes is relatively inexpensive and rapid. There is far less time lag than that experienced with laboratory analytical procedures. Rapid detecting units are valuable for determining whether a hazardous condition exists at a given location at a given time so that workers may be evacuated or suitable protective devices provided. In addition, industrial operators and process engineers need inexpensive and rapidly responsive devices for day-to-day evaluation of the atmospheric levels in a work area.

In evaluating measurements performed with detector tubes, interferences, difficulty in endpoint readings, and possible calibration inaccuracies must all be considered. These factors affect the accuracy of detector tubes. Thus, the use of such tubes must be limited to estimating the concentration of the contaminant.

Portable Direct-Reading Instruments

Several portable direct-reading instruments for the measurement of occupational exposures to nitrogen dioxide are available. With proper calibration and maintenance, they can be more sensitive and accurate than detector tubes, and they provide even more rapid indications of levels of exposure. Such instruments serve useful purposes by providing immediate on-the-spot information as to levels of nitrogen dioxide concentrations in work areas. This makes them particularly useful for evaluating the need

for and effectiveness of control measures. In view of their size and weight, they are not practicable to use for personal sampling. Weights range from about 10 pounds to more than 25 pounds. The instruments do not give information on the levels of any nitric oxide present. Prices of commercially available nitrogen dioxide direct-reading meters are generally in the range of $1,000 to $2,000.

A NIOSH publication [228] has evaluated four commercially available direct reading nitrogen dioxide instruments. The publication discusses such factors and characteristics as portability, ease and simplicity of operation, completeness of instructions in the operation manual, maintenance procedures, direct reading capabilities, cost, ability to operate as a continuous monitor, cost and availability of replacement components, range of concentrations measured, accuracy of manufacturer's calibration, zero drift, response time, stability of calibration setting, ability to record continuously, linearity of response, specificity for nitrogen dioxide, severity of interferences, ease of calibration and zeroing, effects of temperature and humidity, durability, shipping problems, sensitivity, and life of components used to measure nitrogen dioxide.

XII. APPENDIX IV

MATERIAL SAFETY DATA SHEET

The following items of information which are applicable to a specific product or material shall be provided in the appropriate block of the Material Safety Data Sheet (MSDS).

The product designation is inserted in the block in the upper left corner of the first page to facilitate filing and retrieval. Print in upper case letters as large as possible. It should be printed to read upright with the sheet turned sideways. The product designation is that name or code designation which appears on the label, or by which the product is sold or known by employees. The relative numerical hazard ratings and key statements are those determined by the procedures described in Chapter V, Part B, of the NIOSH publication, An Identification System for Occupationally Hazardous Materials. [229] The company identification may be printed in the upper right corner if desired.

(a) Section I. Product Identification

The manufacturer's name, address, and regular and emergency telephone numbers (including area code) are inserted in the appropriate blocks of Section I. The company listed should be a source of detailed backup information on the hazards of the material(s) covered by the MSDS. The listing of suppliers or wholesale distributors is discouraged. The trade name should be the product designation or common name associated with the material. The synonyms are those commonly used for the product, especially formal chemical nomenclature. Every known chemical designation or competitor's trade name need not be listed.

(b) Section II. Hazardous Ingredients

The "materials" listed in Section II shall be those substances which are part of the hazardous product covered by the MSDS and individually meet any of the criteria defining a hazardous material. Thus, one component of a multicomponent product might be listed because of its toxicity, another component because of its flammability, while a third component could be included both for its toxicity and its reactivity. Note that a MSDS for a single component product must have the name of the material repeated in this section to avoid giving the impression that there are no hazardous ingredients.

Chemical substances should be listed according to their complete name derived from a recognized system of nomenclature. Where possible, avoid using common names and general class names such as "aromatic amine," "safety solvent," or "aliphatic hydrocarbon" when the specific name is known.

The "%" may be the approximate percentage by weight or volume (indicate basis) which each hazardous ingredient of the mixture bears to the whole mixture. This may be indicated as a range or maximum amount, ie, "10-40% vol" or "10% max wt" to avoid disclosure of trade secrets.

Toxic hazard data shall be stated in terms of concentration, mode of exposure or test, and animal used, ie, "100 mg/kg LD50-oral-rat," "25 mg/kilo LD50-skin-rabbit," "75 ppm LC man," or "permissible exposure from 29 CFR 1910.1000," or if not available, from other sources of publications, such as the American Conference of Governmental Industrial Hygienists or the American National Standards Institute Inc. Flammable or reactive data could be flash point, shock sensitivity, or other brief data indicating nature of the hazard.

(c) Section III. Physical Data

The data in Section III should be for the total mixture and should include the boiling point and melting point in degrees Fahrenheit (Celsius in parentheses); vapor pressure, in conventional millimeters of mercury (mmHg); vapor density of gas or vapor (air = 1); solubility in water, in parts/hundred parts of water by weight; specific gravity (water = 1); percent volatiles (indicated if by weight or volume) at 70 degrees F (21.1 degrees C); evaporation rate for liquids or sublimable solids, relative to butyl acetate; and appearance and odor. These data are useful for the control of toxic substances. Boiling point, vapor density, percent volatiles, vapor pressure, and evaporation are useful for designing proper ventilation equipment. This information is also useful for design and deployment of adequate fire and spill containment equipment. The appearance and odor may facilitate identification of substances stored in improperly marked containers, or when spilled.

(d) Section IV. Fire and Explosion Data

Section IV should contain complete fire and explosion data for the product, including flash point and autoignition temperature in degrees Fahrenheit (Celsius in parentheses); flammable limits, in percent by volume in air; suitable extinguishing media or materials; special firefighting procedures; and unusual fire and explosion hazard information. If the product presents no fire hazard, insert "NO FIRE HAZARD" on the line labeled "Extinguishing Media."

(e) Section V. Health Hazard Information

The "Health Hazard Data" should be a combined estimate of the hazard of the total product. This can be expressed as a TWA concentration, as a

permissible exposure, or by some other indication of an acceptable standard. Other data are acceptable, such as lowest LD50, if multiple components are involved.

Under "Routes of Exposure," comments in each category should reflect the potential hazard from absorption by the route in question. Comments should indicate the severity of the effect and the basis for the statement if possible. The basis might be animal studies, analogy with similar products, or human experiences. Comments such as "yes" or "possible" are not helpful. Typical comments might be:

> Skin Contact--single short contact, no adverse effects likely; prolonged or repeated contact, mild irritation and possibly some blistering.
>
> Eye Contact--some pain and mild transient irritation; no corneal scarring.

"Emergency and First Aid Procedures" should be written in lay language and should primarily represent first aid treatment that could be provided by paramedical personnel or individuals trained in first aid.

Information in the "Notes to Physician" section should include any special medical information which would be of assistance to an attending physician including required or recommended preplacement and periodic medical examinations, diagnostic procedures, and medical management of overexposed workers.

(f) Section VI. Reactivity Data

The comments in Section VI relate to safe storage and handling of hazardous, unstable substances. It is particularly important to highlight

instability or incompatibility with common substances or circumstances, such as water, direct sunlight, steel or copper piping, acids, alkalies, etc. "Hazardous Decomposition Products" shall include those products released under fire conditions. It must also include dangerous products produced by aging, such as peroxides in the case of some ethers. Where applicable, shelf life should also be indicated.

(g) Section VII. Spill or Leak Procedures

Detailed procedures for cleanup and disposal should be listed with emphasis on precautions to be taken to protect workers assigned to cleanup detail. Specific neutralizing chemicals or procedures should be described in detail. Disposal methods should be explicit including proper labeling of containers holding residues and ultimate disposal methods such as "sanitary landfill" or "incineration." Warnings such as "comply with local, state, and federal antipollution ordinances" are proper but not sufficient. Specific procedures shall be identified.

(h) Section VIII. Special Protection Information

Section VIII requires specific information. Statements such as "Yes," "No," or "If necessary" are not informative. Ventilation requirements should be specific as to type and preferred methods. Respirators shall be specified as to type and NIOSH or US Bureau of Mines approval class, ie, "Supplied air," "Organic vapor canister," "Suitable for dusts not more toxic than lead," etc. Protective equipment must be specified as to type and materials of construction.

(i) Section IX. Special Precautions

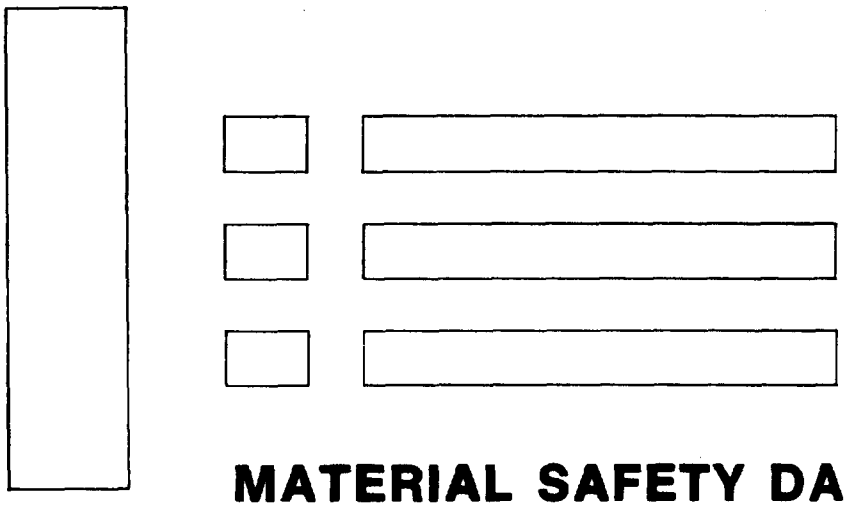
"Precautionary Statements" shall consist of the label statements selected for use on the container or placard. Additional information on

any aspect of safety or health not covered in other sections should be inserted in Section IX. The lower block can contain references to published guides or in-house procedures for handling and storage. Department of Transportation markings and classifications and other freight, handling, or storage requirements and environmental controls can be noted.

(j) Signature and Filing

Finally, the name and address of the responsible person who completed the MSDS and the date of completion are entered. This will facilitate correction of errors and identify a source of additional information.

The MSDS shall be filed in a location readily accessible to workers potentially exposed to the hazardous material. The MSDS can be used as a training aid and basis for discussion during safety meetings and training of new employees. It should assist management by directing attention to the need for specific control engineering, work practices, and protective measures to ensure safe handling and use of the material. It will aid the safety and health staff in planning a safe and healthful work environment and suggesting appropriate emergency procedures and sources of help in the event of harmful exposure of employees.



MATERIAL SAFETY DATA SHEET

I PRODUCT IDENTIFICATION	
MANUFACTURER'S NAME	REGULAR TELEPHONE NO. EMERGENCY TELEPHONE NO.
ADDRESS	
TRADE NAME	
SYNONYMS	

II HAZARDOUS INGREDIENTS		
MATERIAL OR COMPONENT	%	HAZARD DATA

III PHYSICAL DATA			
BOILING POINT, 760 MM HG		MELTING POINT	
SPECIFIC GRAVITY (H_2O=1)		VAPOR PRESSURE	
VAPOR DENSITY (AIR=1)		SOLUBILITY IN H_2O, % BY WT	
% VOLATILES BY VOL		EVAPORATION RATE (BUTYL ACETATE=1)	
APPEARANCE AND ODOR			

IV FIRE AND EXPLOSION DATA

FLASH POINT (TEST METHOD)		AUTOIGNITION TEMPERATURE			
FLAMMABLE LIMITS IN AIR, % BY VOL.	LOWER		UPPER		
EXTINGUISHING MEDIA					
SPECIAL FIRE FIGHTING PROCEDURES					
UNUSUAL FIRE AND EXPLOSION HAZARD					

V HEALTH HAZARD INFORMATION

HEALTH HAZARD DATA

ROUTES OF EXPOSURE

INHALATION

SKIN CONTACT

SKIN ABSORPTION

EYE CONTACT

INGESTION

EFFECTS OF OVEREXPOSURE

ACUTE OVEREXPOSURE

CHRONIC OVEREXPOSURE

EMERGENCY AND FIRST AID PROCEDURES

EYES

SKIN

INHALATION

INGESTION

NOTES TO PHYSICIAN

VI REACTIVITY DATA

CONDITIONS CONTRIBUTING TO INSTABILITY

INCOMPATIBILITY

HAZARDOUS DECOMPOSITION PRODUCTS

CONDITIONS CONTRIBUTING TO HAZARDOUS POLYMERIZATION

VII SPILL OR LEAK PROCEDURES

STEPS TO BE TAKEN IF MATERIAL IS RELEASED OR SPILLED

NEUTRALIZING CHEMICALS

WASTE DISPOSAL METHOD

VIII SPECIAL PROTECTION INFORMATION

VENTILATION REQUIREMENTS

SPECIFIC PERSONAL PROTECTIVE EQUIPMENT

RESPIRATORY (SPECIFY IN DETAIL)

EYE

GLOVES

OTHER CLOTHING AND EQUIPMENT

IX SPECIAL PRECAUTIONS

PRECAUTIONARY
STATEMENTS

OTHER HANDLING AND
STORAGE REQUIREMENTS

PREPARED BY _____

ADDRESS _____

DATE _____

XIII. TABLES AND FIGURES

TABLE XIII-1

PROPERTIES OF NITRIC OXIDE AND NITROGEN DIOXIDE

	Nitric Oxide NO	Nitrogen Dioxide NO2
Formula weight	30.01	46.01
Density (gas)	1.3402	1.4494
Melting point C	-163.6	-11.2
Boiling point C	-151.8	21.2
Solubility per 100 cc hot water (60 C)	2.37 cc	----
cold water (0 C)	7.34	soluble, decomposes

Derived from Handbook of Chemistry and Physics [1]

TABLE XIII-2

OXIDATION RATE OF NITRIC OXIDE IN AIR (20% O2) AT 20 C

Concentration (ppm)	Oxidation Time		
	25%	50%	90%
10,000	8.4 sec	24 sec	3.6 min
1,000	1.4 min	4 min	36 min
100	14 min	40 min	6 hours
10	2.3 hours	7 hours	63 hours
1	24 hours	72 hours	648 hours

From Austin [167]

TABLE XIII-3

NITROGEN OXIDES FROM CUTTING WITH OXYACETYLENE TORCH

Sample No.	Collection Time (A.M.)*	Oxides of Nitrogen (ppm)		
		NO2	NO	NO2 + NO
1	6:55	25	165	190
2	6:55	--	--	210
3	7:06	--	--	260
4	7:11	--	--	300
5	7:18	--	--	290
6	7:20	90	180	270
7	7:21	--	--	300
8	7:27	--	--	330
9	7:27	--	--	310
10	7:28	--	--	340

*Cutting began at 6:40 A.M.

From Norwood et al [23]

TABLE XIII-4

FORMATION OF OXIDES OF NITROGEN BY VICTOR TORCH

Sample No.	Torch Characteristics	Gas Evolution Rate (mg/min)		
		Nitrogen Dioxide	Nitric Oxide	Total
1m	Flame only	16	215	231
2m	Flame only	--	---	250
3m	Stainless-steel melting	--	---	47
5m	Stainless-steel melting	9	62	71
6m	Carbon steel cutting*	14	150	164

*More oxygen is used during cutting than during melting

From Norwood et al [23]

TABLE XIII-5

NITROGEN DIOXIDE CONCENTRATIONS FROM FLAME-CUTTING

Minutes after Completion of Cut	Mean Concentration of Nitrogen Dioxide (ppm)			
	Flame Only	Unprimed Plate	Polyamide-Cured Primer	Amine Adduct-Cured Primer
1	594	86	82	95
2	518	90	99	97
3	493	68	78	91
4	465	78	87	67
5	437	70	93	91
6	382	74	79	68
7	346	62	82	81
8	333	64	77	67
9	308	62	77	72
10	288	68	70	59
15	196	45	48	60
20	100	30	32	37

From Steel and Sanderson [19]

TABLE XIII-6

CONCENTRATION OF NO, AND CO2 IN SILO

Days after Onset of Filling Silo		1	2	3	4	5	6	7	9	11	13	21	42
No. of Loads of Silage		12	18	22	32	48	64	90	93	93	93	93	93
Sampling sites													
Periphery of tower													
1 ft above	NO ppm	0	0.4	0.6	1	2	1	300	5	0.6	0.1		0.2
surface	NO2 ppm	0	1	2	2	9	1	280	5	0.6	0.1		0.0
	CO2 %v/v	2	4	2	1	2	0.4	41	0.1	0.0	0.0		0.4
5 ft above	NO ppm				0.5	2	0.3	150	5	0.4			
surface	NO2 ppm				1	5	0.6	220	4	0.4			
	CO2 %v/v				0.1	2	0.0	40	0.1	0.0			
6 in. below	NO ppm								395	10	0.6	0.6	
surface	NO2 ppm								200	1	0.2	0.6	
	CO2 %v/v								39	13	11	2	
Center of tower													
1 ft above	NO ppm	0	9	240	240	630	560	3					
surface	NO2 ppm	0	0.1	400	220	1920	360	2					
	CO2 %v/v	19	34	49	49	60	78	0.2					
5 ft above	NO ppm	0	3	29	3	30	3	2					
surface	NO2 ppm	0	0.2	24	4	35	4	2					
	CO2 %v/v	3	23	12	3	25	3	0					
6 ft below	NO ppm										4.5	0.2	
surface	NO2 ppm										0.1	0.0	
	CO2 %v/v										222	8	

Load covered with polyethylene sheet after 6th day, removed before tests on 11th day

From Commins et al [29]

TABLE XIII-7

SUMMARY OF EPIDEMIOLOGIC AND EXPERIMENTAL STUDIES ON HUMAN EXPOSURE TO NITROGEN DIOXIDE

Concentration in ppm	Length of Exposure	Type of Exposure	Observed Effects	Remarks	Reference
38-345	Working lifetime	Occupational: Shotfiring operations in coal miners	Forced Expiratory Volume (FEV 0.75) and vital capacity reduced, residual volume and total lung capacity increased relative to controls	Inappropriate control sample. Exposure to high levels of carbon monoxide and carbon dioxide in addition to "nitrous fumes"	Kennedy [28]
62-158	3 separate exposures ranging from 10 min to 2 hrs	Experimental: Continuous inhalation	62 ppm for 1 hour: Laryngeal irritation, but no other effects. 25-100 ppm for 2 hours: Marked mucosal irritation, increased pulse and respiratory rates. 158 ppm for 10 minutes: Coughing, irritation of nasal and laryngeal mucosa lacrimation, headache, nausea, and vomiting. No delayed or long-term illness	Probable exposure to nitric oxide and airborne nitric acid in addition to nitrogen dioxide	Lehman & Hasegawa [39]
4-20	Acute, duration not stated	Occupational: Open arc welding	Conjunctivitis and pharyngitis which subsided 18 hrs after exposure	Exposure to oxides of nitrogen	Morley & Silk [63]
2.0-10.3	Unknown	Occupational: Arc welding	Slight increase in methemoglobin levels in blood	Exposure to oxides of nitrogen	McCord et al [56]
4-5	10 min	Experimental: Continuous inhalation	Decrease in effective lung compliance with corresponding increase in expiratory and inspiratory maximum viscous response	5 healthy adult male subjects	Abe [67]
0.0-5.0	30 breaths or 15 min	Experimental: Continuous inhalation.	Exposure at 1.5-5.0 ppm increased airway resistance. Significant decrease in arterial oxygen tension, and significant increase of end-expiratory arterial pressure at 4-5 ppm. No effects noted below 1.5 ppm	88 chronic bronchitis patients	Von Nieding et al [68]
0.5-5.0	15-60 min	Experimental: Continuous inhalation	Significant reduction in carbon monoxide diffusing capacity in 16 healthy male subjects exposed for 15 min at 5 ppm. Significant decrease in arterial oxygen partial pressure with corresponding increase in alveoloarterial oxygen pressure gradients in 14 chronic bronchitis patients exposed for 15 min at 5 ppm. Continued exposure to 60 min did not significantly change findings at 15 min. Increased airway resistance in 70 chronic bronchitis patients exposed at and above 1.5 ppm		Von Nieding et al [69]

TABLE XIII-7 (CONTINUED)

SUMMARY OF EPIDEMIOLOGIC AND EXPERIMENTAL STUDIES ON HUMAN EXPOSURE TO NITROGEN DIOXIDE

Concentration in ppm	Length of Exposure	Type of Exposure	Observed Effects	Remarks	Reference
0.4-2.7	4-6 years	Occupational: Chemical works	Complaints of sporadic cough, mucopurulent expectoration, and dyspnea on exertion. Normal chest X-ray, spirometry, and blood pH. Carbon dioxide partial pressure and total carbonic acid in blood increased. Significant decrease in serum proteins and significant increase in urinary amino acids and glycoproteins	Conflicting results on the presence of chronic obstructive pulmonary disease. Total lack of environmental data	Kosmider et al [71]
Less than 2.8	Unknown	Occupational: Printing shop and sulfuric acid plant	Dental erosion and gingivitis; emphysema and pulmonary tuberculosis; cardiovascular hypotonia and bradycardia; polycythemia rubra, granulocytosis, basophilia; decreased osmotic fragility of red blood cells, accelerated agglutination of the blood cells; reduced catalase index, reduced alkali reserve, reduced blood sugar	Workers probably exposed to sulfuric acid mists and sulfur dioxide at unknown concentrations	Vigdortschik et al [70]
Low Exposure= 0.106 High Exposure= 0.711	24 hrs/day	Community: Ambient air near TNT plant	Higher incidence of acute respiratory disease in high exposure community compared with low exposure community, particularly in children below age 12. No difference in chronic respiratory disease between communities	Suspended nitrates and total suspended particulates higher in high exposure community compared with other communities. Concentrations of sulfur dioxide and other contaminants not reported	French [74]

TABLE XIII-8

SUMMARY OF CLINICAL AND EPIDEMIOLOGIC STUDIES ON HUMAN EXPOSURE TO NITRIC OXIDE

Concentration in ppm	Length of Exposure	Type of Exposure	Observed Effects	Remarks	Reference
#112	3 min	Anesthesia accident	One patient showed signs of cyanosis and methemoglobinemia, followed 18 1/2 hours later by death. Autopsy indicated severe pulmonary edema. Second patient showed signs of cyanosis, but recovered fully following proper medical treatment.	Accidents due to contamination of nitrous oxide by nitric oxide, the analysis of which was not described.	Clutton-Brock [58]
#3	Working lifetime	Occupational: Nitrogen fertilizer production	Exposed workers had higher carboxy - and methemoglobin levels in their blood compared with controls. Exposed workers developed pyroxidine deficiency.	Exposure to carbon monoxide, ammonia, and mixed oxides of nitrogen	Nizhegorodov & Markhotskii [57]
2-10	Unknown	Occupational: Arc-welding	Slight increase in methemoglobin levels	Exposure to mixed oxides of nitrogen	McCord et al [56]

TABLE XIII-9

SUMMARY OF EFFECTS OF EXPOSURE TO NITRIC OXIDE
IN EXPERIMENTAL ANIMALS

Concentration in ppm	Species	Duration of Exposure	Type of Exposure	Observed Efects	Reference
5000-20000	Dog	Up to 50 min	Continuous	5000 ppm: Decreased arterial oxygen tension, rise in methemoglobin and arterial carbon dioxide tension. If exposure greater than 24 min, death occurred 7-120 min after exposure. 20000 ppm: Death in 15-50 minutes	Greenbaum et al [81]
2500-5000	White mice	Up to 12 min	"	Animals exposed at 5000 ppm died after 6-8 min. Animals exposed at 2500 ppm died after 12 min of exposure.	Flury and Zernik [86]
310-3500	"	Up to 8 hrs	"	LC50 = 320 ppm All animals survived an 8-hr exposure at 310 ppm. At high concentrations, nitric oxide 4 times more toxic than nitrogen dioxide	Pflesser [47]
175-2100	Mice, guinea pig	Up to 6 hrs	"	Mice exposed at 2100 ppm for 30 min produced 80% methemoglobin. Exposure at 322 ppm for 6 hrs produced 60% methemoglobin. No change in recovery of resting respiratory rhythm in guinea pigs at 175 ppm for 120-150 min	Paribok and Grokholskaya [87]

TABLE XIII-10

SUMMARY OF EFFECTS OF EXPOSURE TO NITROGEN DIOXIDE IN EXPERIMENTAL ANIMALS

Concentration in ppm	Species	Duration of Exposure	Type of Exposure	Dependent Variable(s)	Results	Reference
88-1445	Rats	2-240 min	Continuous	Mortality	Time / LC50: 2 min 1445 ppm; 5 " 833 "; 15 " 420 "; 30 " 174 "; 60 " 168 "; 240 " 88 "	Gray et al [89]
115-416	Rats, dogs, guinea pigs	5-60 min	"	"	Time / LC50: Rat: 5 min 416 ppm; 15 " 201 "; 30 " 162 "; 60 " 115 "; Guinea Pig: 15 min 315 ppm. Threshold of toxicity approximately 25% of LC50 levels for rats. At these levels, dogs showed no gross or microscopic changes, rats showed some pulmonary edema.	Carson et al [90]
12.5-100	Rats	Until animals died or arbitrary termination of exposure	Continuous 24 hrs/day, 7 days/wk	Microscopic changes in pulmonary system	Exposure at 100 ppm resulted in death within 24 hrs. Rats exposed at 12.5 ppm had moderate hypertrophy and hyperplasia of bronchial and bronchiolar epithelium as well irregular alveolar ducts and alveoli after 40 days of exposure.	Freeman and Haydon [97]
20-70	Guinea pigs	30 min	Continuous	Antigen sensitization	Exposure at 70 ppm enhanced sensitization, 40 ppm and less did not.	Matsumura [122]
50	Hamsters	1-10 wks	Intermittent: 21-23 hrs/day	Microscopic changes in lung tissue	1/3 of animals died within first 3 days. Epithelial hyperplasia and hypertrophy of bronchial and alveoli noted in animals killed immediately after exposure. Regression of inflammatory and epithelial hyperplastic changes observed in animals killed 4 wks after termination of exposure.	Kleinerman and Cowdrey [91]

TABLE XIII-10 (CONTINUED)

SUMMARY OF EFFECTS OF EXPOSURE TO NITROGEN DIOXIDE IN EXPERIMENTAL ANIMALS

Concentration in ppm	Species	Duration of Exposure	Type of Exposure	Dependent Variable(s)	Results	Reference
30-50	Guinea pigs	30-45 min	Continuous	Mortality due to inhaled acetylchlorine	Exposure at 50 ppm resulted in significantly higher mortality in animals pretreated with nitrogen dioxide than in controls. No differences in mortality between controls and pretreated groups at lower concentrations of nitrogen dioxide	Matsumura et al [123]
5-50	Rabbits	3 hrs	"	Phagocytic activity	Supression of virus-induced resistance and phagocytic activity	Acton and Myrvik [121]
10,22,36,45	Rats	Single 4-hr periods	"	Microscopic changes in tracheal and lung tissue	Normal trachea and lungs 4-8 days after exposure	Diggle and Gage [94]
15 and 40	Guinea pigs	Continuous for 10 wks or interrupted for 4 1/2 hrs	15 ppm-cont. 40 ppm-int.: 1/2 hr every 2 hrs for 4 1/2 hrs	Oxygen consumption of tissue homogenates	No increase in lung tissue, but marked increase in liver tissues	Buckley and Balchum [112]
8-40	Rabbits	3 hrs	"	Cellular distribution in lung tissue	Significant increase in intraalveolar heterophiles from exposure at 8 ppm	Gardner et al [110]
40	Mice	6-8 wks	"	Oxygen consumption and LDH activity in lung	Increase in oxygen consumption and LDH activity at sites of nitrogen dioxide lung lesions	Buckley and Loosli [114]
4-30	Mice	14 days at 4-7 ppm, 24 hrs at 30 ppm.	"	Lung capillary permeability and epithelial cell damage	Leakage of tritriated serum into pulmonary lavage fluid	Sherwin and Richters [115]
26	Dogs	191 days	"	Macro- and microscopic changes in pulmonary system	1 dog showed bullous emphysema. Others showed a striking increase in the firmness of the lungs and emphysema, microscopically.	Lewis et al [101]
20-25	Rats, rabbits, guinea pigs	3 wks-18 mon	Intermittent 2 hrs/day, 3-4 days/wk	Macro- and microsco pulmonary changes	Changes judged equivalent to microbullous emphysema observed in guinea pigs exposed for 15-18 mon. No such changes observed in rats or rabbits.	Kleinerman and Wright [96]

TABLE XIII-10 (CONTINUED)

SUMMARY OF EFFECTS OF EXPOSURE TO NITROGEN DIOXIDE IN EXPERIMENTAL ANIMALS

Concentration in ppm	Species	Duration of Exposure	Type of Exposure	Dependent Variable(s)	Results	Reference
15-25	Rats, guinea pigs, rabbits	2-hr exposures for 1 or 5 days	Continuous	Macro- and microscopic pulmonary changes	Pulmonary edema noted after one 2-hour exposure. Repair noted 2 wks after exposure. Edema and inflammation less severe after multiple 2-hr exposures than to single 2-hr exposure. Degree of morphologic change related to exposure concentration	Kleinerman and Wright [95]
25	Mice	4 1/2 mon	Intermittent: 30 min/day, 5 days/wk	Microscopic changes of lung tissue due to exposure to nitrogen dioxide alone and to carbon particles with absorbed nitrogen dioxide	Lung lesions such as destruction of alveolar walls was apparent in animals exposed to combined carbon-nitrogen dioxide. No lesions noted in animals exposed only to nitrogen dioxide	Boren [124]
25	Dogs	6 mon	Continuous	Macro- and microscopic changes in pulmonary system	1 dog showed macroscopic bullous emphysema. All dogs showed enlargement of alveoli	Riddick et al [100]
2-25	Rats	Natural lifetime except for 1 experiment in which rats were sacrificed at daily intervals during the 1st week of exposure at 18 ppm	"	Microscopic changes of pulmonary system and lung weights	Terminal bronchiolar epithelial hypertrophy was observed to begin on the 5th day of exposure at 18 ppm. Widespread hypertrophy of respiratory epithelium indicative of emphysema resulted from continuous exposure at 10-25 ppm. Exposure at 2 ppm resulted in a reduction of bronchiolar cilia, inhibition of normal exfoliation and blebbing of epithelial cells, and appearance of cytoplasmic crystalloid inclusions.	Freeman et al [99]

TABLE XIII-10 (CONTINUED)

SUMMARY OF EFFECTS OF EXPOSURE TO NITROGEN DIOXIDE IN EXPERIMENTAL ANIMALS

Concentration in ppm	Species	Duration of Exposure	Type of Exposure	Dependent Variable(s)	Results	Reference
1-25	Dogs, mice, rabbits, guinea pigs, rats, and hamsters	Up to 18 mon	Intermittent: 6 hrs/day, 5 day/wk	Macro- and microscopic changes of the pulmonary system	Dogs exposed at 1 ppm for 1 year had moderately dilated alveolar ducts and sacs which contained some edematous fluid and an occassional macrophage. After 18 mon of exposure some thickening of alveolar septa and chronic inflammatory cells were noted. Hamsters exposed at 25 ppm for 3-6 mon showed minor changes in bronchiolar epithelium. No changes noted in rabbits and mice exposed for up to 18 mon at 5 and 25 ppm, respectively	Wagner et al [93]
15-17	Rats	48 hrs	Continuous	Macrophage division	Large increase in no. of dividing macrophages, as well as total no. of macrophages.	Evans et al [111]
2 and 17	"	1 hr - 43 days	"	Microscopic changes in lung tissue	Increased lung weight and severe injury to bronchiole epithelium in animals exposed at 17 ppm. Animals exposed at 2 ppm showed no increase in lung weights compared with controls. Loss of cilia, hypertrophy, and focal hyperplasia noted after 3 days of exposure. Tissue recovery observed in animals killed after 21 days of exposure	Stephens et al [130]
2 and 17	"	Up to 360 days	"	Microscopic changes in bronchioles and terminal alveoli	Increased cell proliferation during the first 3-5 days, returning to normal after this time	Evans et al [131]
5-16	Dogs and rabbits	1 hr	"	Microscopic changes of capillary endothelium and alveolar epithelium	Exposure had greatest effect on capillary endothelium. Findings included bleb formation, endothelial cell organelles in the capillary lumens, and appearance of platelets and polymorphonuclear leukocytes in lumens of capillaries adjoining blebs.	Kilburn and Dowell [104]

TABLE XIII-10 (CONTINUED)

SUMMARY OF EFFECTS OF EXPOSURE TO NITROGEN DIOXIDE IN EXPERIMENTAL ANIMALS

Concentration in ppm	Species	Duration of Exposure	Type of Exposure	Dependent Variable(s)	Results	Reference
3-16	Dogs	1 hr	Continuous	Microscopic changes of endothelial cells	Bleb formation, loss of pinocytic vesicles, and mitochondrial swelling. Exposure at 3 ppm resulted in bleb formation without other changes.	Dowell et al [105]
15 ± 2	Rats	1, 2, & 7 days	"	Ultrastructural changes of lung tissue	Bronchiolar epithelium was less columnar, brush cells increased in number, microvilli became smaller, and number of macrophages increased.	Parkinson and Stephens [107]
15	Guinea pigs	3 mon	"	Quantitative change in alveolar cells	Both an increase in the number of alveolar cells and the number of cells per alveolar space resulted from exposure.	Sherwin et al [108]
5-15	"	1 year	5 ppm: 4 hrs/ day, 5 days/wk 15 ppm: 7 1/2 hrs/ day, 5 days/wk	Antibody titers	Minimal microscopic change of lung tissue. Serum antibodies appeared within 160 hrs, and increased with continued exposure.	Balchum et al [116]
15	Rats	Natural life-time	Continuous	Pulmonary changes	Animals had voluminous dry lungs, microscopic signs of epithelial hypertrophy emphysema, and loss of cilia.	Freeman et al [102]
15	Guinea pigs	26-40 days	23 hrs/day	Enzyme activity in lung	Decrease in aerobic isozyme and increase in anaerobic isozyme in lung tissue.	Buckley and Balchum [113]
1-14.8	Mice	1.9-14.8 ppm for 4 hours and 1, 2.3, 6.6 ppm for 17 hrs	Continuous	Antibacterial activity of animals infected with radiophosphorus labeled Staphylococcus aureus	Decreased bactericidal activity in animals infected then exposed to 7 ppm. Exposure at 2.3 ppm for 17 hrs prior to infection also resulted in reduced bactericidal response.	Goldstein et al [139]
9.3-14.3	Rats	10-24 days	Intermittent: 4 hrs/day, 5 days/wk	Pulmonary changes	Immediately after exposure, rats showed severe rhinitis and tracheitis with less severe pneumonitis. Animals killed 8 wks after exposure showed signs that the inflammatory process had subsided. However, localized areas of emphysema were noted.	Gray et al [92]

TABLE XIII-10 (CONTINUED)

SUMMARY OF EFFECTS OF EXPOSURE TO NITROGEN DIOXIDE IN EXPERIMENTAL ANIMALS

Concentration in ppm	Species	Duration of Exposure	Type of Exposure	Dependent Variable(s)	Results	Reference
0.5-14	Mice	Ct = 7; continuous at 0.5, 1.5, 3.5 ppm; 7 hrs/day at 3.5 ppm for up to 288 hrs	Continuous and intermittent	Mortality due to challenge by Streptococcus pyogenes	Ct was not a constant. Lower mortality with intermittent exposure. Linear regression of % mortality versus exposure time significantly different from zero slope for exposure at 0.5 ppm, not so for exposure at 1.5 ppm	Coffin et al [140]
8-12	Rabbits	3-4 mon	Continuous	Microscopic changes of pulmonary system	Emphysema-like dilations of peripheral alveoli were noted.	Haydon et al [98]
0.5-12	Monkeys, dogs, rabbits, guinea pigs, rats	90 days	"	Hematologic changes, weight gain, gross lung pathology	Bronchitis, bronchopneumonitis, pneumonia, and foci of multinucleated cells noted in animals exposed at 12 ppm. No lung pathology observed in animals exposed at and below 5 ppm	Steadman et al [145]
10	Guinea pigs	6 wks	"	Ultrastructural changes of lung tissue	Thickening of blood-gas barrier by replacement of ultrathin type 1 cells by cuboidal or columnar type 2 pneumocytes.	Yuen and Sherwin [106]
10	"	7 wks	"	Macrophage congregation	Exposed animals showed an increase in macrophage congregation as well as an increase in the number of macrophages/epithelial cell.	Sherwin et al [109]
5-10	Squirrel monkeys	5 ppm: 2 mon 10 ppm: 1 mon	"	Susceptibility to infection	Increased susceptibility to infection by K. pneumonia and influenza virus	Henry et al [117]
5	Rats, mice, monkeys	90 days	"	Mortality	No significant mortality. No remarkable changes in growth or blood chemistry	MacEwen and Geckler [128]
5	Squirrel monkeys	169 days	"	Antibody production due to intratracheal injections of mouse-adapted influenza virus	Hemaglutination-inhibition antibody not affected. Serum neutralizing antibody increased initially, but no differences between experimental and control animals by 169th day.	Fenters et al [137]

TABLE XIII-10 (CONTINUED)

SUMMARY OF EFFECTS OF EXPOSURE TO NITROGEN DIOXIDE IN EXPERIMENTAL ANIMALS

Concentration in ppm	Species	Duration of Exposure	Type of Exposure	Dependent Variable(s)	Results	Reference
Mean = 4.5	Rats, mice, monkeys	90 days	Continuous	Hematologic and urinary changes as well as microscopic changes of the liver, kidneys, lungs, heart, pancreas, spleen, adrenals, cortex, medulla, and spinal cord	Mortality was low. Reduced weight gain, but no other significant pathologic findings	House [127]
4	Rats, mice, guinea pigs	6 mon	Intermittent: 4 hrs/day, 5 days/week.	Incidence of pulmonary obstructive disease	No significant difference between experimental and control groups	Gray et al [126]
0.8–4	Rats	16 wks	Continuous	Macro- and microscopic changes of lung tissue	No macroscopic signs of chronic obstructive disease. Only minimal microscopic changes	Haydon et al [143]
2.5 and 3.5	Mice	2 hrs	"	Susceptibility to Klebsiella pneumoniae	Increased susceptibility at 3.5 ppm, not at 2.5 ppm.	Purvis and Erlich [135]
1.5, 2.5, 3.5	"	"	"	Mortality due to challenge by Klebsiella pneumoniae	Significant increase at 3.5 ppm, but not at 2 lower levels.	Erlich et al [136]
0.5–3.5	"	2 hrs and 9 mon	Continuous or Intermittent (6 hrs/day, 5 days/wk)	Mortality resulting from exposure to airborne Klebsiella pneumoniae	No effect following 2-hr exposure at 3.5 ppm. Significant increase in mortality in animals exposed continuously for 3 mon or intermittently for 1 mon at 0.5 ppm	Ehrlich [155]
2.9 ± 0.71	Rats	9 mon	24 hrs/day, 5 days/wk.	Changes in lung weights and physiology	12.7% mean increase in lung weights. 13% mean decrease in lung compliance. Reduction of surface-active properties of lung-wash fluid	Arner and Rhodes [134]
2 ± 1	"	Natural lifetime	Continuous	Changes in respiratory function as well as microscopic changes of lung tissue	Persistant tachypnea in all animals. No changes in airflow resistance or dynamic compliance. Microscopic changes including reduced blebbing of cytoplasm into airways, loss of cilia, and appearance of intracytoplasmic crystalloid inclusions.	Freeman et al [129]
2	Guinea pigs	1, 2, or 3 wks	"	Ratios of lactate dehydrogenase-positive wall cells to alveoli	Exposed animals showed changes of LDH activity suggesting increases in type 2 pneumocytes as compared with controls	Sherwin et al [132]

TABLE XIII-10 (CONTINUED)

SUMMARY OF EFFECTS OF EXPOSURE TO NITROGEN DIOXIDE IN EXPERIMENTAL ANIMALS

Concentration in ppm	Species	Duration of Exposure	Type of Exposure	Dependent Variable(s)	Results	Reference
0.1-2	Mice	3 1/2-7 mon	Continuous at 0.5 and 2 ppm. Intermittent: 0.5 ppm with 1-hr peaks at 2 ppm, 5 days/wk, or 0.1 ppm with 3-hr peaks of 1 ppm, 5 days/wk	Cellular morphology of lungs, phagocytic activity and oxygen consumption of alveolar macrophages	Cell counts, macrophage viabilities at isolation, and oxygen consumption of macrophages unaffected. In vitro phagocytic activity reduced in animals exposed intermittently at 0.5/2 ppm for 3 1/2 or 7 mon. No such change noted in animals exposed continuously at 2 ppm. Changes in morphology of macrophages noted in animals exposed intermittently at 0.5/2 ppm. No such changes observed in other exposure groups	Aranyi and Port [147]
0.5-2.0	"	Up to 40 wks	Continuous at 2.0 ppm or intermittent (5 days/wk) at 0.5 ppm with 1-hr peaks at 2.0 ppm (0.5/2)	Immune response	No difference between experimental and control animals in HI antibody titers. SN titers significantly depressed in animals exposed at 0.5/2. Significant increase in IgA, IgM, IgG, and IgG2 immunoglobin levels in animals exposed to nitrogen dioxide, particularly in those animals exposed at 0.5/2 ppm	Erlich et al [158]
1-1.5	"	1 Mon	Continuous	Microscopic changes in trachea and lungs	Desquamative bronchitis observed in animals killed immediately after exposure. Infiltration of lymphocytes seen in lungs of animals killed 1 and 3 months after exposure. No controls	Chen et al [133]
1	Guinea pigs	180 days	8 hrs/day	Macro- and microscopic changes in the lung. Hematologic, urinary, and immunologic changes	Evidence of chronic respiratory disease such as bronchitis bronchopneumonia, extravasation of blood in lungs, and foci of emphysema. Urinary hydroxproline and acid mucopolysaccharides were increased. Decreased serum proteins, immunoglobulins, and weight gain	Kosmider et al [71]

TABLE XIII-10 (CONTINUED)

SUMMARY OF EFFECTS OF EXPOSURE TO NITROGEN DIOXIDE IN EXPERIMENTAL ANIMALS

Concentration in ppm	Species	Duration of Exposure	Type of Exposure	Dependent Variable(s)	Results	Reference
1.0	Rabbits	1 hr	Continuous	Changes in protein structure of lung tissue	Peak shift in absorbance spectrum in animals killed immediately after exposure. Absorbance spectrum returned to normal in animals killed 24-48 hrs after exposure	Bueli et al [151]
1.0	Rats	1-6 days	4 hrs/day	Changes in lung lipid structure	Absorption spectra indicative of dienne conjugation	Thomas et al [153]
1.0	Squirrel monkeys	493 days	Continuous	Microscopic changes in lung tissue and immune responses resulting from challenge with A/PR18/34 virus	No difference between experimental and control animals in hemaglutination-inhibition antibody titers, body temperatures, respiratory function, body weights, hematologic values, and ultrastructural changes. Monkeys exposed to nitrogen dioxide produced serum neutralization antibody within 21 days of exposure as well as signs of chronic pulmonary obstructive disease by the end of exposure.	Fenters et al [157]
0.5-1.0	Rats	1 hr at 1 ppm, 4 hrs at 0.5 ppm	"	Changes in mast cells of lung	Exposure at 1 ppm resulted in loss of cytoplasmic granules, rupture, and reduction in number of mast cells. Exposure at 0.5 ppm for 4 hours resulted in degranulation of mast cells.	Thomas et al [152]
0.2-1.0 in combination with 0.2-2.0 ppm. nitric oxide.	Dogs	4 1/2 years	16 hrs/day	Cardiovascular changes	No significant effects	Bloch et al [149]
0.1-1.0 in combination with 0.1-2.0 ppm nitric oxide	"	18 mon	"	Pulmonary function	No change in single-breath carbon monoxide diffusing capacity, dynamic pulmonary compliance, or total pulmonary resistance.	Vaughn et al [148]
0.8	Rats	Natural lifetime	Continuous	Respiratory physiology and microscopic changes of lung tissue	Sustained tachypnea 20% above controls. Minimal morphologic changes. No gross or microscopic signs of obstructive disease	Freeman et al [144]

TABLE XIII-10 (CONTINUED)

SUMMARY OF EFFECTS OF EXPOSURE TO NITROGEN DIOXIDE IN EXPERIMENTAL ANIMALS

Concentration in ppm	Species	Duration of Exposure	Type of Exposure	Dependent Variable(s)	Results	Reference
0.5	Mice	3-12 mon	6, 18, 24 hrs/day	Alveolar size	Lung alveoli expanded in all mice exposed to nitrogen dioxide as compared with controls	Blair et al [146]
0.5	"	1-12 mon	Continuous or intermittent (6 or 18 hrs/day)	Mortality, rate of bacterial clearance, serum lactic dehydrogenase resulting from exposure to Klebsiella pneumoniae	Reduced rate of clearance. LDH showed shift from anaerobic to aerobic bands. Significant increase in mortality in animals continuously exposed for 3 mon or longer, and in animals intermittently exposed for 6 mon	Ehrlich and Henry [156]
0.4	Guinea pigs	1 wk	Continuous	Protein level in lung lavage fluid	Animals exposed to nitrogen dioxide showed higher protein levels in lung lavage fluid than controls	Sherwin and Carlson [154]

TABLE XIII-11

INCIDENCE OF PULMONARY TUMORS IN NO2 EXPOSED AND IN CONTROL MICE

Duration of Exposure (MO)	5 ppm NO2		Control		% Difference of Tumor Incidence in Exposed versus Control Animals
	No. with Tumors / No. Examined	% Tumor Incidence	No. with Tumors / No. Examined	% Tumor Incidence	
12	7/10	70	4/10	40	+30
14	7/15	47	8/15	53	-6
16	15/24	62	15/24	62	0

From Wagner et al [93]

TABLE XIII-12

CORRELATION OF OXIDES OF NITROGEN WITH TORCH SIZE

Size of Tip	Acetylene Consumption (cu ft/hr)(1)	Time After Ignition of Torch (minutes)	Concentration of Nitrogen (as NO2) (ppm)	Average Concentration (ppm)
#4	15.9	1	25	
		3	50	
		4	40	38
#6	31.6	1	65	
		3	75	
		4	100	80
#8	60.0	1	150	
		2	210	
		3	240	
		4	240	210
#10	88.5	1	210	
		2	270	
		3	320	
		4	320	280
#12	175	1	240	
		4	370	
		5	430	
		7	370	352

(1) Rated capacity of tip

From Adley [11]

TABLE XIII-13

OXIDES OF NITROGEN IN LARGE, VENTILATED COMPARTMENTS

Volume of Compartment (cu ft)	Remarks	Number of Samples	Oxides of Nitrogen (Expressed as NO2)	
			Average Concentration (ppm)	Maximum Concentration (ppm)
7200	Operator shrinking intermittently. Fair natural ventilation.	5	48	89
8700	Operator working on deck plates adjacent to fresh air supply hose.	5	19	32
8700	Operator shrinking in compartment having one fresh air supply hose about 20 feet away.	3	34	38
9000	Operator shrinking near outside hatch. Good natural ventilation.	3	4	4
10000	Two operators shrinking. Two fresh air supply hoses introducing a total of about 1,000 cfm.	12	17	27

Note: #10 torch tips being used during all sampling.

From Adley [11]

TABLE XIII-14

NITROGEN OXIDES EXPOSURES FROM WELDING OPERATIONS IN SEVEN SHIPYARDS

Location		Total Number Samples	Nitrogen Oxides (ppm)						
Major	Minor		0-4	5-9	10-14	15-19	20-24	25 or over	Mean
Hull	Inner bottoms	172	98	51	18	1	1	3	5.2
	Fore- and after-peaks and small tanks	166	90	43	22	7	3	1	5.8
	Cargo holds, superstructure and other large spaces	661	364	180	68	22	13	14	5.9
	Top deck and outside shell	104	58	23	10	6	1	6	7.3
Sub-assembly	Inner bottoms	48	22	11	5	4	1	5	8.9
	Fore- and after-peaks	136	87	34	11	2	1	1	4.6
	Superstructure	69	34	24	4	7	0	0	5.8
	Open, flat sheets	257	170	59	13	10	3	2	4.7
Shop	Fabrication	295	176	67	39	9	3	1	5.2
	Pipe	111	56	30	11	3	6	5	7.4

From Dreessen et al [13]

FIGURE XIII - 1. NITROGEN DIOXIDE/NITRIC OXIDE SAMPLING TUBE

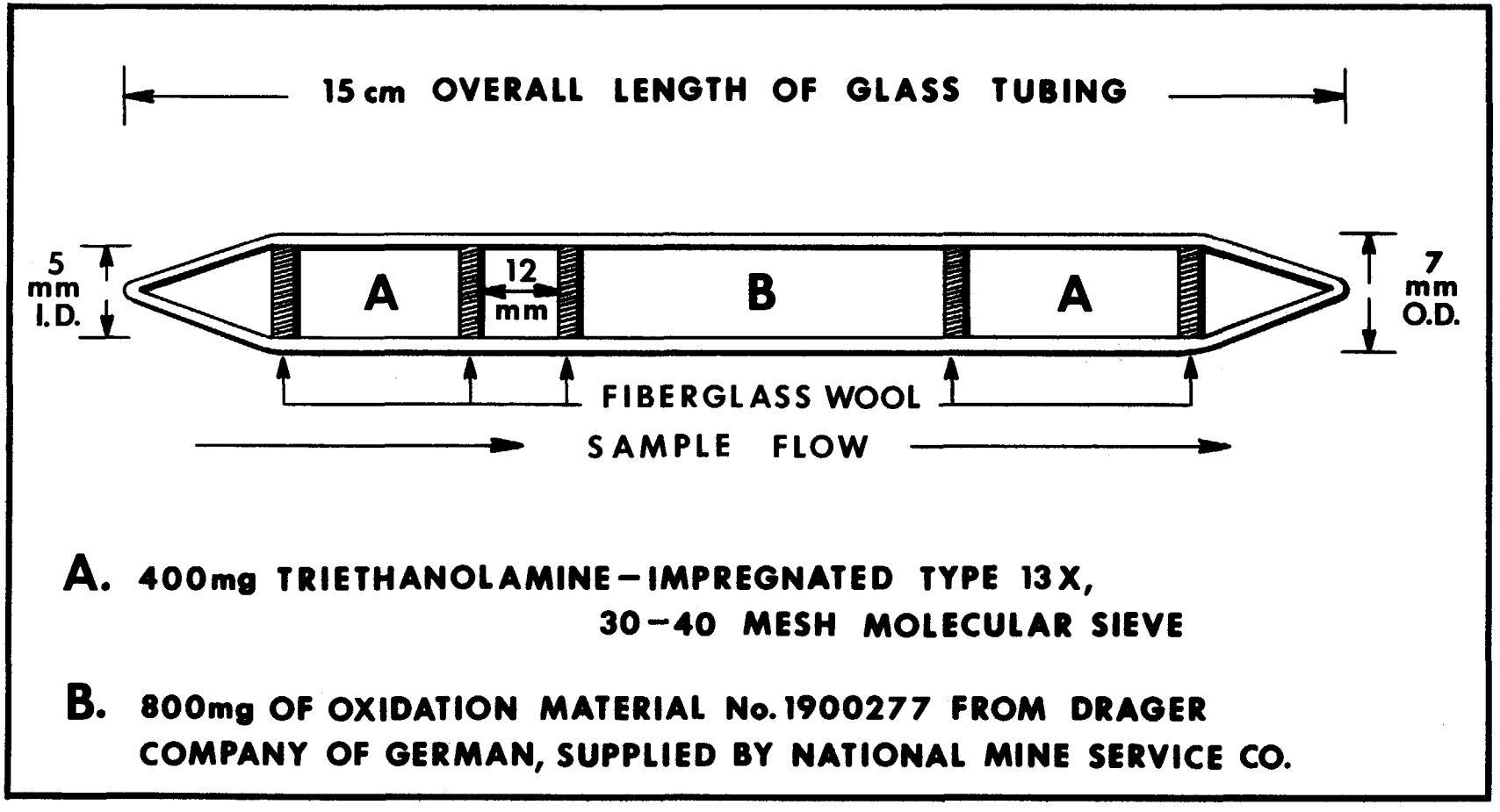
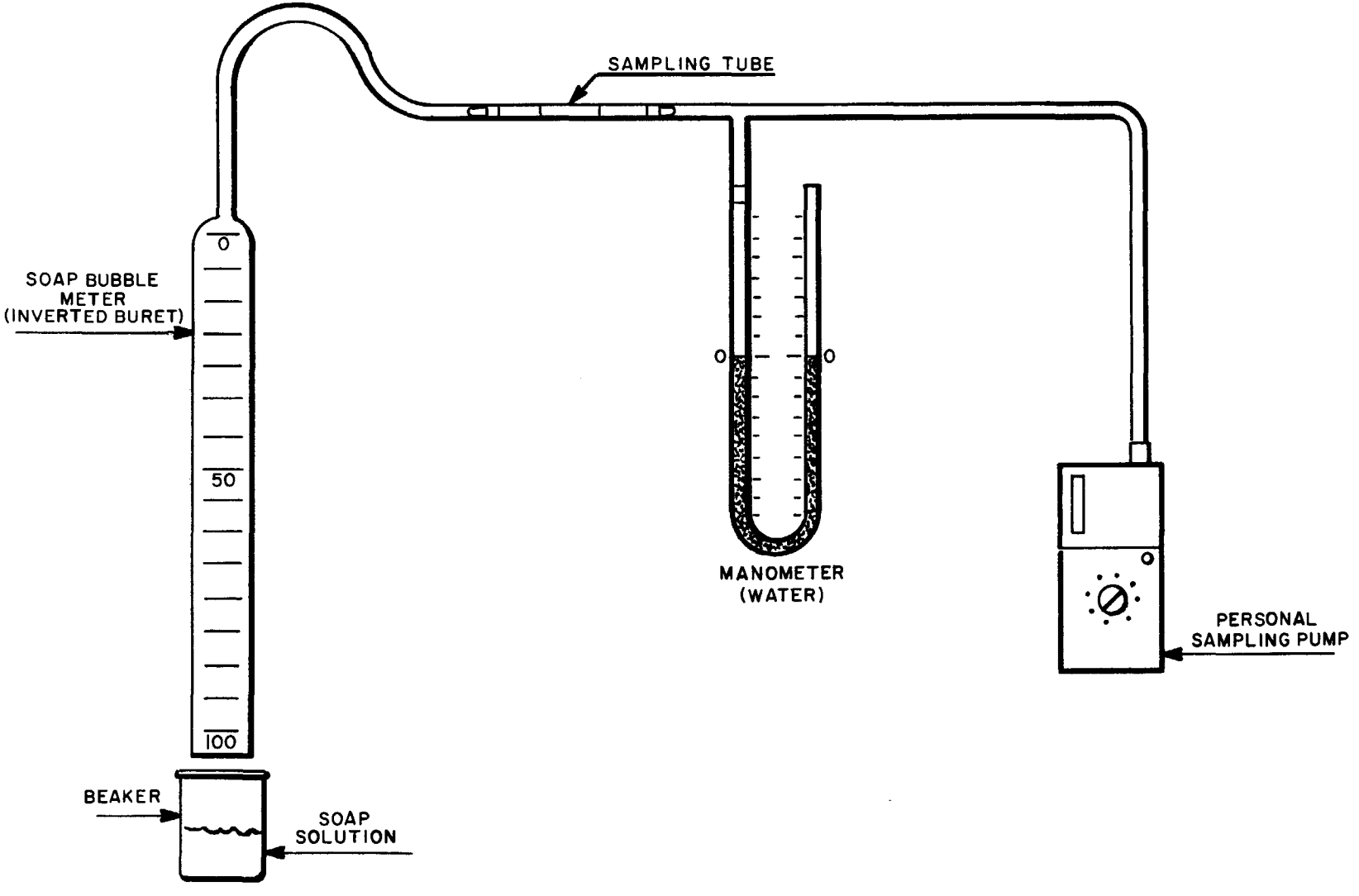


FIGURE XIII - 2. CALIBRATION SCHEME FOR PERSONAL SAMPLING PUMP AND SAMPLING TUBE



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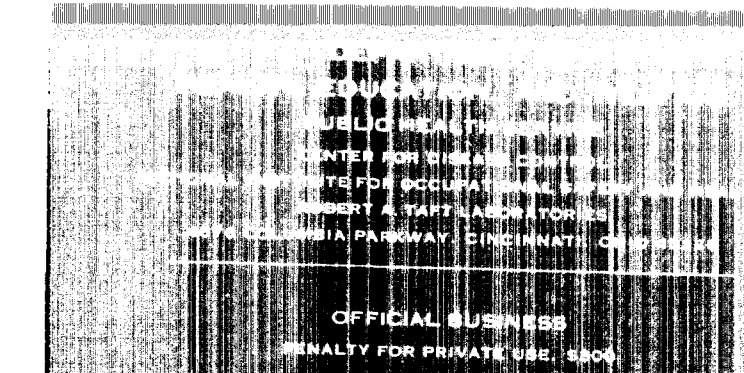

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