Evaluation of Biological Significance of Nitrogen Oxides Exposure

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This paper attempts to assess health risks associated with nitrogen oxides (NOx) exposures. Experimental animal studies show that continuous or intermittent exposures to concentration of 0.04 to 0.5 ppm nitrogen dioxide (NO2) produce a number of pulmonary and systemic effects. Controlled human exposure studies show that short-term exposures to 0.3 to 0.5 ppm NO2 or 1.0 ppm nitric oxide produce bronchoconstriction and blood biochemical changes in some subjects. When we consider together both findings of the animal and human exposure studies, we may suggest with some reliability that the concentrations of NO2 in ambient and indoor air pollutions may have adverse health effects on some subjects, and may partly contribute to the respiratory symptoms or illness and reduced pulmonary function observed in the epidemiological studies.

(Key Words: Nitrogen oxides, Health effects)

INTRODUCTION

Indoor air quality could be an important factor affecting human respiratory health since most people might spend more than 70% of their time indoors. Indoor exposure can constitute an important fraction of the total exposure to many environmental pollutants. Among various indoor pollutants, nitrogen oxides (NOx) from combustion appliances and tobacco smoke are considered as having important effects on respiratory health. The range of observed hourly indoor NOx concentrations is 0.03 to 0.5 ppm with a maximum of about 1.0 ppm for both nitric oxide (NO) and nitrogen dioxide (NO2) without external ventilation. Several recent studies (1–5) of the health status of children and adult living in homes with gas ranges have shown that they had more respiratory symptoms or illness and poorer respiratory function than children and adult living in comparable home with electric ranges, although Keller et al. (6) have failed to find an association between gas-cooking emissions and adverse respiratory health effects. Comstoke et al. (7) found a significant increase of risk for respiratory problems such as chronic coughing and impaired lung function for adults having a history of exposure to gas cooking. Although NO2 was considered as the most likely causal agent, Florey et al. (8) could not establish a significant relation between the prevalence of respiratory illness or the degree of reduced lung function and measured NO2 concentrations. This paper attempts to assess health risks associated with NO and NO2 exposures.

CURRENT KNOWLEDGE OF CONTROLLED HUMAN EXPOSURE STUDY

Controlled human exposure studies provide necessary information for evaluating what kinds of pollutants and how much the pollutants contribute to the acute effects observed in the epidemiological studies, since they can clearly demonstrate relationships between specific pollutants exposure and short-term health effects. Hackney et al. (9) reported a significant decrease of forced vital capacity in 16 normal subjects after 2-hr exposure to 1.0 ppm NO2.

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Folinsbee et al. (10) found no significant change in pulmonary function in 15 normal subjects after 2-hr exposure to 0.62 ppm NO2. On the other hand, Kerr et al. (11) reported a significant decrease of quasistatic compliance in 10 normal subjects after 2-hr exposure to 0.5 ppm NO2. Kagawa et al. (12) reported a significant increase of functional residual capacity (FRC) after 2-hr exposure to 0.5 ppm NO2 and a significant decrease of specific airway conductance (Gaw/Vtg) after 2-hr exposure to 0.3 ppm NO2. Hazucha et al. (13) reported a slight but not significant increase of specific airway resistance (sRaw) in 15 normal subjects after 1-hr exposure to 0.1 ppm NO2.

In subjects with asthma and chronic bronchitis considered at least partially representative of the sensitive population to air pollutants, Kerr et al. (11) reported significant increases in FRC, total lung capacity and residual volume in 20 subjects with asthma and chronic bronchitis after 2-hr exposure to 0.5 ppm NO2. Seven of 13 subjects with asthma complained of some degree of chest tightness, burning of eyes, headache, or dyspnea with exercise after exposure to NO2. Orehek et al. (14) reported a significant increase of sRaw in 13 of 20 asthmatics after 1-hr exposure to 0.1 ppm NO2. Hazucha et al. (13) reported a slight but not significant increase of sRaw after 1-hr exposure to 0.1 ppm NO2.

The effect of NO2 exposure on bronchial reactivity may be a sensitive index in detecting responses to low concentrations of NO2. In normal subject, no significant increase of bronchial reactivity was observed after 2-hr exposure to 0.3 ppm NO2 (15) and 0.5 ppm NO2. In asthmatic subject, Kleinman et al. (16) reported approximately two-thirds of 31 asthmatic subjects showed some increase of bronchial reactivity with 0.2 ppm NO2 exposure for 2-hr. Orehek et al. (14) showed the increased bronchial reactivity in 13 of 20 asthmatic subjects after 1-hr exposure to 0.1 ppm NO2. However, Hazucha et al. (13) could not find a similar reaction in a similar study.

The inconsistency among the NO2 health effects results should not be surprising, since this inconsistency might result from the choice of pulmonary function tests, individual variation in reactivity including biological adaptation to smoking, passive smoking and chronic air pollutants exposure, exercise level, race and nutritional differences and environmental factors. Although the experimental human exposure studies on NO2 health effects under 1.0 ppm show no consistent exposure-effect relationship from the view-point of the effects on pulmonary function, the existing evidence may suggest the variable physiological effects in normal subject after 2-hr exposure with intermittent light exercise to 0.5 ppm NO2 and more measurable effects in sensitive subject.

There are a few reports of the effects of NO2 exposure on blood biochemistry. Posin et al. (17) reported a significant decrease in the activity of the erythrocyte membrane enzyme acetylcholinesterase in 10 normal subjects after 2.5 to 3-hr exposure to 1.0 ppm NO2. Kagawa et al. (18) reported a significant increase of plasma histamine after 2-hr exposure to 0.3 and 0.5 ppm NO2 in 7 normal subjects. They also observed a significant decrease of glutathione (GSH) in 15 normal subjects after 2-hr exposure to 0.3 and 0.5 ppm NO2 (unpublished data). However, Chaney et al. (19) reported a significant increase of GSH in 19 normal subjects after 2-hr exposure to 0.2 ppm NO2.

NO is emitted from a wide range of common combustion process. The amount of NO2 produced initially by these combustion processes is usually small compared with that of NO formed. The NO/NO2 ratio is usually more than 2. The maximum concentration of NO ranges between 0.3 to 1.0 ppm in the unvented enclosed indoor environment from the common combustion process such as gas or oil stoves or gas oven and the combustion of tobacco products. However, little attention is given to the effects of NO on human health. There is only one controlled human exposure study of the effects of NO under 1.0 ppm. Kagawa (20) showed the 2-hr exposure to 1.0 ppm NO produced a significant decrease of Gaw/Vtg in 8 normal subjects and his recent study showed a significant decrease of GSH in 6 normal subjects (unpublished data).

The existing evidence shows the exposure to 0.5 ppm NO2 or 1.0 ppm NO for 2-hr with intermittent light exercise will produce the significant effects on pulmonary function and blood biochemistry. The significance of these findings depends on whether these findings constitute an adverse health effects in relation to the develop-
ment of respiratory symptoms and illness.

CURRENT KNOWLEDGE FROM ANIMAL EXPOSURE STUDY

The most interesting response of animal to NO₂ exposure may be increased susceptibility to respiratory infection. Voisin et al. (21) demonstrated NO₂ affected bactericidal activity such as phagocytosis by alveolar macrophages. Thirty min to 2-hr exposure of cultured rabbit alveolar macrophages to 0.1 ppm NO₂ resulted in decreased bactericidal activity. Ehrlich et al. (22) reported significant differences in mortality from experimentally induced bacterial pneumonia were observed in mice after 90 days exposure to 0.5 ppm NO₂. Motomiya et al. (23) also observed a similar effect in mice after 3 months exposure to 0.3 to 0.5 ppm NO₂, and these mice exhibited enhanced pathologic responses including a greater incidence of adenomatous proliferation of bronchial epithelial cells.

The zone between alveolar and terminal bronchiolar airways has been demonstrated to be a sensitive one to NO₂ exposure, since the moderately low solubility of NO₂ will give a higher penetration to lower airways. Sherwin et al. (24) reported an increase of protein of lung lavage in guinea pigs exposed to 0.4 ppm NO₂ for one week and they (25) reported hyperplasia and hypertrophy of alveolar type II cells in mice exposed to 0.34 ppm NO₂, 6-hr/day, 5 days/week for 6 weeks. Buell (26) reported the isolation of swollen, damaged, insoluble collagen fibers from the lungs of rabbits exposed to 0.25 ppm NO₂ for 4-hr/day, 5 days/week for 24 days. Modifications of collagenous tissue may be reflected in the increased excretion of collagen degradation products such as hydroxyproline (HOP) in the urine. Kasahara et al. (27) reported an increase of urinary HOP in rats exposed to 0.5 ppm NO₂ for 30 days and 1.0 ppm NO₂ for one week. Port et al. (28) reported substantial variations in pore size and as many as 10 pores per alveolus suggesting the development of emphysema in mice exposed to 0.1 ppm NO₂ continuously for 6 months except for 2-hr per day, during which "peak" exposures of 1.0 ppm. The recent morphological observation under electron microscope by Takenaka et al. (29) has revealed morphological alteration of alveolar wall at an extremely lower concentration. They reported a tendency of the increase of arithmetic mean thickness (AMT) of the alveolar wall in rats exposed to 0.04 ppm NO₂ for 18 months and larger increase of AMT in rats exposed to 0.04 ppm NO₂ for 27 months.

An important mechanism of tissue damage caused by NO₂ is considered to be the peroxidation of tissue molecules. NO₂ initiates lipid peroxidation, which subsequently causes cell injury or death and the symptoms associated with NO₂ exposure. Sagai et al. (30) reported that ethane exhalation in breath as an index of lipid peroxidation increased significantly in rats following 0.04 ppm NO₂ exposure for 9 and 18 months. The formation of lipid peroxidation may relate to the thickening of the alveolar wall.

As an another interesting report, Suzuki et al. (31) showed a decrease of arterial blood oxygen tension (Pao₂) of rats exposed to 0.4 ppm NO₂ for 9 months. The decrease of Pao₂ may be induced by the thickness of the alveolar wall. These animal exposure studies suggest that small animals exposed to such 0.04 to 0.5 ppm as concentrations of NO₂ observed in ambient and indoor air pollution show the significant biochemical, physiological and morphological changes.

EVALUATION OF BIOLOGICAL SIGNIFICANCE OF NO₂ EXPOSURE

The major problem on interpretation of the animal and human laboratory tests is to evaluate how much NO₂ contributes to the health effects observed in the epidemiological studies. Most animal and human exposure studies have been conducted by diluting pure NO₂ in filtered clean air. These studies may not actually reflect the effect of equivalent concentrations of NO₂ in the environment, where complex chemical interaction may take place with other pollutants and meteorological factors. The extrapolation of animal toxicological findings to human health is always a difficult problem, since animals have different biochemical, functional and morphological aspects, and different life span from human being. However, we might predict health risk in human population if we could find some similar changes in quality and quantity among the health effects from the animal and human exposure studies.
Experimental animal studies showed that continuous or intermittent exposure of small animals to concentrations of 0.04 to 0.5 ppm NO₂ produced a number of pulmonary or systemic responses. Controlled human exposure studies showed that short term exposure to 0.3 to 0.5 ppm NO₂ or 1.0 ppm NO produced bronchoconstriction and blood biochemical changes in some subjects. The increased bronchial reactivity after exposure to 0.1 or 0.2 ppm NO₂ was reported in asthmatic subjects, which suggested the possibility of increased permeability of the epithelium damaged by peroxidation of the unsaturated lipids of the epithelial cell membrane. The observed results of the human exposure study might suggest that the similar findings as the animal toxicological findings would be observed in some subjects when they were exposed repeatedly to such concentrations observed indoors as 0.3 to 0.5 ppm NO₂ for a long time. Further, it should be reminded that there were other respiratory irritants in the presence of such concentration of NO₂. If links could be established between acute and chronic effects observed in animals, and also between acute effects in animal and human exposure studies, one could then rationaly predict what chronic effects were likely in humans receiving long-term ambient and indoor exposures.

When we consider together both findings of the animal and human exposure studies as shown in Figure 1, we may suggest with some reliability that NO₂ exposure may partly contribute to the respiratory symptoms or illness and reduced pulmonary function observed in the epidemiological studies, and that concentrations of NO₂ in ambient and indoor air pollutants may have adverse effects on some subjects.

REFERENCES
21) Voinis C, Aerts C, Houdlet JL, Tonnell AB and Romon
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24) Sherwin RP and Carlson DA: Protein content of lung lavage fluid of guinea pigs exposed to 0.4 ppm nitrogen dioxide. Arch Environ Health 27: 90–93, 1973

Health Effects of Nitrogen Oxides

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Animal Exposure Study

- 0.04 to 0.5ppm NO2
- 1 week to 9 months exposure

In reactive animals

- Morphological, Biochemical and Functional Changes
  - Hyperplasia of alveolar type II cells
  - Increase of thickness of alveolar wall
  - Swollen collagen fibers
  - Bronchiolitis
  - Increase of ethane exhalation in breath
  - Increase of protein of lung lavage
  - Increase of urinary hydroxyproline
  - Decrease of PaO2

- Increase of Susceptibility to Airborne Infection

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Human Exposure Study

- 0.3 to 0.5ppm NO2
- 1.0ppm NO
- 2 hours exposure

In reactive subjects

- Pulmonary Function and Biochemical Changes
  - Bronchoconstriction
  - Increase of plasma histamine
  - Decrease of glutathione

- 0.1 to 0.2ppm NO2
- 1 to 2 hours exposure

In some asthmatic subjects

- Increase of Bronchial Reactivity

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Epidemiological Study

In reactive subjects

- Respiratory symptoms and illness
- Reduced pulmonary function
- Increase of urinary hydroxyproline

Fig. 1 Evaluation of Biological Significance of Nitrogen Oxides Exposure