Toxicology of ozone as characterized by laboratory animals and extrapolated to humans

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ABSTRACT

The adverse effects of ozone have been amply demonstrated in animal toxicology, human clinical, and epidemiology studies. Each of these research approaches has various strengths and weaknesses, but together the coherence is remarkable. This paper interprets the effects of ozone as demonstrated and inferred from laboratory animal toxicology studies. The major classes of ozone effects are: decrements in pulmonary function and symptoms, respiratory morbidity, inflammation, alterations of host defenses, and chronic effects on lung structure and function. All have been observed in laboratory animal studies, except for those calling for 'uniquely' human events (e.g., the reporting of symptoms or admission to hospitals). However, the findings in animals are often more extensive because a wider range of endpoints can be used. This raises the issue of whether such findings can be quantitatively extrapolated to humans. Most experts accept the premises supporting the qualitative extrapolation. That is, if ozone causes the effect in several animal species, it likely could cause the effect in humans, albeit at an unknown exposure. The quantitative extrapolation (i.e., the estimate of exposure that is likely to cause the effects in humans) is more controversial. This paper discusses the

principal results of animal inhalation toxicology studies that greatly expand our understanding of the effects of ozone and provides a preliminary estimate of the potential for humans to experience chronic effects. The predominant topics relate to the impacts of several days of exposure and chronic exposure for months to years. Human clinical studies suggest that the pulmonary function of people accommodates to repeated intermittent ozone exposure. A similar pattern of attenuation of pulmonary function is shown in laboratory animals; however, cellular damage continues. This has been confirmed in humans. Chronic exposures of rats and monkeys result in structural remodeling of the lung, especially in the region where the conducting airways and gas exchange region meet. Various changes are involved, which many experts interpret as being consistent with incipient peribronchiolar fibrogenesis within the interstitium of the lung. In both rats and monkeys, such structural changes persisted after exposure ceased. Furthermore, seasonal exposure caused equivalent or sometimes greater effects than continuous exposure in both species. Using an extrapolation model with numerous assumptions and exposure data from a hypothetical child who plays outdoors and an outdoor worker exposed to ozone over a 214-day season in New York city, rat and monkey lung structural studies suggest potential chronic health effects of ozone. The combined use of animal and human data as linked by quantitative extrapolation has not only refined the assessment of the health impact of ambient ozone exposure but has established a viable paradigm to assess the health effects of other potentially harmful air pollutants.

1. INTRODUCTION

Controlled laboratory animal studies were the first to show the potential for ozone to cause a range of health effects. Even today animal studies provide unique insights into health effects because the exposures are controlled and the endpoints invasive. For example, rats have been exposed to known concentrations of ozone for several years and their lungs have been autopsied with advanced scientific methods. Even though laboratory animal studies can provide insights unavailable in human studies, there is

considerable uncertainty in extrapolating the results of animal toxicology studies to humans. Humans in the ambient environment are exposed to imprecisely known concentrations of ozone in combination with other pollutants, and there are interspecies differences in the lungs and in the impact of ozone on them. The optimal risk assessment strategy for ozone (and other pollutants) thus involves an integrated interpretation of evidence from animal toxicology, human clinical, and epidemiology studies. This enables the strengths of each discipline to contribute to the overall assessment. The health effects database for ozone is one of the best, facilitating such an integration; analogous effects are observed with all three of these research approaches.

Public health concerns for ozone center on effects on pulmonary function and symptoms, respiratory morbidity, inflammatory responses, effects on host defenses, and chronic effects on the respiratory tract. Animal toxicology studies have directly enabled and expanded understanding of all these classes of effects, except for respiratory morbidity (e.g., increased hospital admissions for respiratory causes in relation to increased ozone concentrations). The goal of this paper is to summarize this contribution of laboratory animal research by focusing on the quantitative extrapolation of such studies to humans. The information to be summarized here is drawn from the ozone criteria document by the U.S. Environmental Protection Agency [1], and readers wanting more detailed information should consult that document containing numerous references. Only a very few critical papers are cited in this paper.

Effects of ozone observed in several animal species are very likely to be observed in humans given the substantial similarities between species and the commonality of the mechanism(s) of action of ozone. This qualitative extrapolation has been recognized and accepted for some time. Although the molecular mechanism(s) of action of ozone is not known fully, molecular targets (e.g., carbon-carbon double bonds, sulfhydryl groups) are identical across species. However, there are significant interspecies differences which cast uncertainty on the quantitative extrapolation. Thus, even those who agree on the qualitative extrapolation argue about what exposures cause effects in humans. Recent scientific advances in quantitative extrapolation, discussed in the ozone criteria document, expand the understanding of several effects, especially chronic effects.

2. ACUTE AND SHORT-TERM EFFECTS

Ozone affects host defenses. Alveolar macrophages, which remove particles and microbes from the alveolar region by phagocytosis, have less ability to engulf microbes after exposures to ozone as low as 0.1 ppm (2 hours) in rabbits [2] and 0.08 ppm (6.6 hours, moderate exercise) in humans [3]. One of the most investigated indicators, susceptibility to lung bacterial infection in mice, is increased after a 3-hour exposure to 0.08 ppm [4]. There is no direct corollary in humans, but given the interspecies similarity of the mechanisms of antibacterial lung host defenses, impacts on human host defenses can be hypothesized. Impaired clearance also has implications for interactions with other pollutants, especially particulate matter. Rats exposed to an urban pattern of ozone for 6 weeks had increased retention of asbestos fibers in the lung tissue 30 days after exposure ceased [5], suggesting an impact of ozone on the biological impact of the known carcinogen, asbestos.

The variety of studies of the lung and systemic immune system show mixed effects, but the database is not yet robust enough for clear conclusions. The effects of ozone on antiviral defenses are uncertain. There is no evidence yet of effects on the early course of viral lung infections, but laboratory animal studies suggest an increase in postinfluenza lung damage after a few months of exposure.

Pulmonary function decrements in humans are one of the hallmarks of ozone exposure. Many chamber, field, and epidemiology studies have shown that ozone decreases forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV_1). These changes primarily result from inhibition of inspiration that is thought to be caused by neurogenic and/or inflammatory mechanisms. This response has been observed in healthy subjects exposed to levels as low as 0.08 ppm for 6.6 hours while undergoing moderate exercise [6]. Somewhat higher concentrations cause an increase in breathing frequency in humans. Several species of animals have similar shifts to rapid, shallow breathing after ozone exposure. Figure 1 demonstrates the commonality of rat and human changes in FVC after ozone exposure at different exercise levels. As can be observed here (and in all other exercise studies), a concomitant increase in exercise increases the degree and harmonization of response in humans and rats.

Reports of attenuation of ozone-induced pulmonary function changes and symptoms with several days of exposure (exposures for 1 to 3 hours/day) began to appear in 1977 [9]. The body of work clearly indicates that the spirometric responses of humans are observed on the first 1 to 4 days of a series of intermittent exposures to ozone. At higher ozone concentrations, greater responses may be seen on Day 2. However, as exposures are repeated on the next few days, responses decreased or were absent. Further research in humans showed that responsiveness returned at about 1 or 2 weeks after the original series of exposures. Symptom changes paralleled the functional effects. Because environmental exposures to elevated levels of ozone often persist for a few days, interpretation of the impacts of these findings on risk was important. Such an interest stimulated animal toxicology studies. Tepper and co-workers [10] found that the pulmonary function of rats had a similar pattern of attenuation to ozone exposure. Even so, lung remodeling progressed, and signs of inflammation (lavageable protein) were sustained over the 5-day exposure period. In humans, some inflammatory markers attenuate and some persist; damage, as indicated by a biochemical marker, persists. Joint interpretation of these studies results in a conclusion that risk persists, and perhaps is even enhanced, even though pulmonary function changes revert towards normal. Perhaps there is a linkage. As breathing returns to normal, more ozone may penetrate to the more distal regions of the lung, which are thought to be more vulnerable.

One of the earliest observations of the effects of ozone was the production of edema in the lungs of animals exposed acutely to high concentrations. Years of follow-up in animal studies showed that ozone affected the barrier function of the lung. This function is central to health because the barrier must allow air exchange, while keeping microbes and other undesirable environmental elements out of the body and keeping essential fluids and cells in the body rather than flooding the air spaces. This barrier function is also linked to inflammation, which for the lung, is commonly characterized by an increase in polymorphonuclear leukocytes (PMN's), protein, and other bioactive compounds in lung lavage fluid.

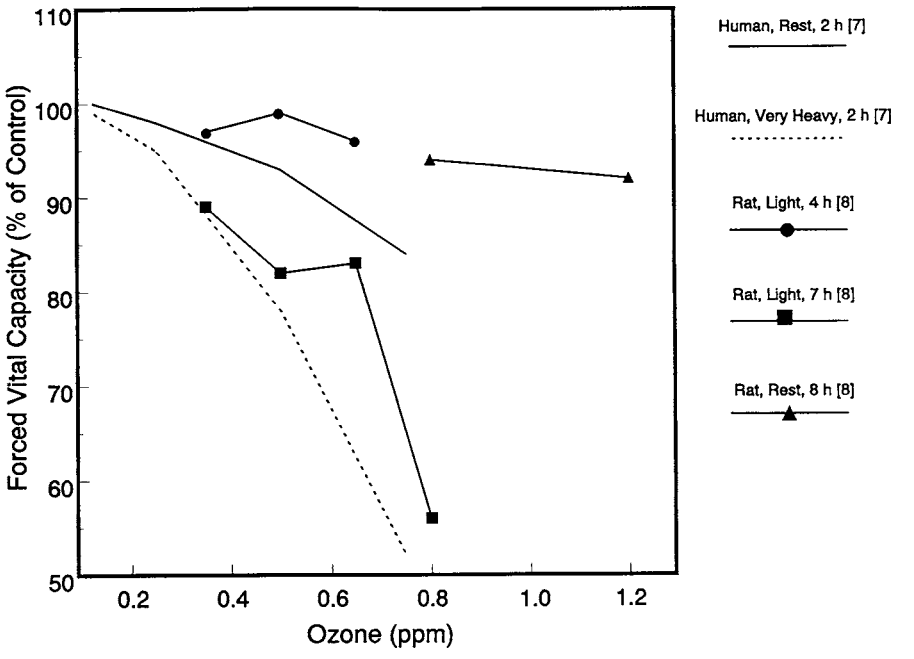


Figure 1. Effects of Ozone on Forced Vital Capacity in Rats and Humans (shaded area is predicted range of changes in humans expected between light exercise [top line] and very heavy exercise [bottom line].) The key also indicates whether the rats were exposed at rest or under light exercise [1].

Numerous animal toxicology studies have been conducted on these endpoints in search of better understanding of the impacts of such changes. Such research stimulated human clinical studies [3] that found that 6.6-hour exposures of healthy humans during moderate exercise increased the PMN's (0.08 ppm), bioactive mediators (0.08 ppm), and protein (0.1 ppm) in lung lavage fluid. Figure 2 compares the protein in bronchoalveolar lavage fluid in three species of animals and in humans as a function of pulmonary tissue dose of ozone. Briefly, Miller and co-workers [11] used dosimetry models to calculate the dose to the pulmonary region (i.e., alveolar region) as a function of concentration and duration of exposure, exercise level, and species. The pulmonary region was chosen because it likely is the site of damage that allows protein to enter the air spaces. They observed a remarkable similarity in the dose-response across species. Additional studies

in laboratory animals and humans exposed to ozone have shown that inflammatory responses persist for about a day after exposure ceases. Inflammation is of concern because it is evidence that injury has occurred. Even though there are many uncertainties, a role of repeated inflammation in the causation of chronic lung disease cannot be ruled out.

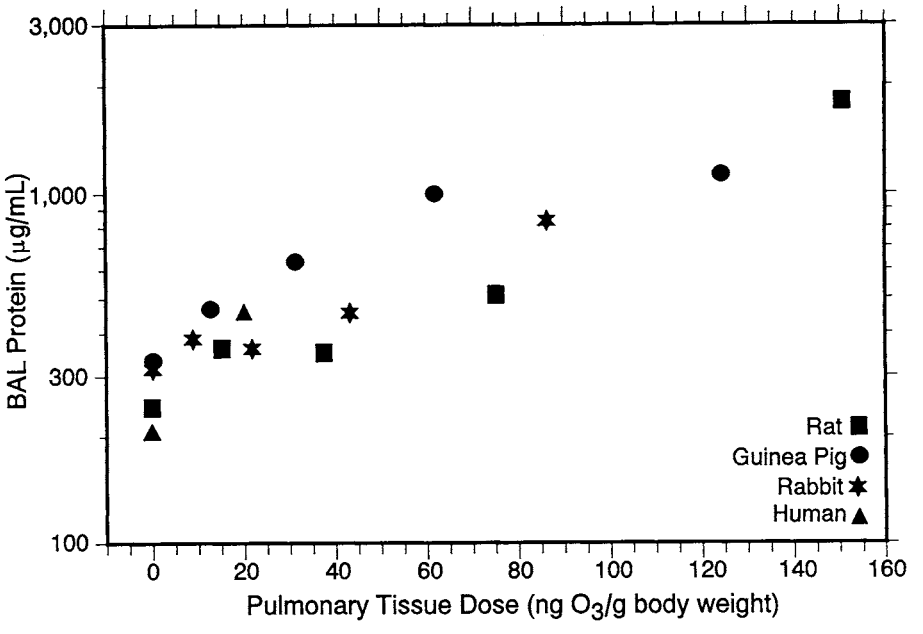


Figure 2. Protein in the bronchoalveolar lavage (BAL) for several laboratory animal species and humans, as related to the estimated pulmonary dose (normalized per gram of body weight) [1,11].

3. CHRONIC EFFECTS OF OZONE

3.1. The Nature of the Effects

The few studies of the immune system or antibacterial host defenses after chronic exposure of laboratory animals either demonstrated no effects or did not increase the magnitude of effects caused by short-term exposure. Thus, lung structural and functional changes are of most concern after months to years of exposure to ozone. At present,

most knowledge about the effects of chronic exposures is derived from laboratory animals. Epidemiology studies have not been performed with the ability to discern such chronic effects in humans, assuming they exist. Even so, the epidemiological evidence that exists is qualitatively supportive of lung function decrements in people living in more highly polluted communities.

Research findings in several species of animals, including nonhuman primates, are remarkably similar. Structural changes occur along the whole respiratory tract, but those in the small airways and in the centriacinar regions (where the conducting airways and the gas exchange region join) are of greater health concern because of the importance of these areas to gas exchange, the primary function of the lungs. Figure 3 provides a schematic of the types of changes that occur. Figure 4 contains a description of the time course of the response during and after exposure.

As duration of exposure increases from days to months, a pattern emerges. Inflammatory exudate usually peaks in the first few days of exposure, resulting in increased permeability and the movement of cells into the air spaces. This response falls off in a few days, remaining at a low level as exposure continues and attaining recovery when exposure stops. Epithelial hyperplasia increases over the first few days and then plateaus, dropping off after exposure ceases. In this process, ciliated cells (that move debris and other material up and out of the lung) are sloughed off and replaced with nonciliated cells. Within the centriacinar region, there is hypertrophy of the epithelial cells of the proximal alveolar region of the transition zone of the alveoli and smallest airways while interstitial fibroblasts increase and create an exudate; collagen fibers accumulate; and the interstitium thickens. Even after exposure to ozone ceases, collagen can continue to accumulate. Several of these changes are similar to the earliest lesions found in human respiratory bronchiolitis, some of which may progress to fibrotic lung disease. Functional changes have been sought in several studies, but have been variable. Most pulmonary function tests do not measure changes in small airways and the centriacinar region. Even so, some studies suggest 'stiffer' lungs, which are consistent with fibrotic-like changes.

The influence of different exposure patterns is complex. For example, Tyler et al. (1988) exposed monkeys to a 'daily' regimen (8 hours/day, 7 days/week, 18 months) or a 'seasonal' regimen (same as for daily, but only every other month, for a total of

9 months of exposure over an 18-month period). Both groups of monkeys were affected (e.g., both had respiratory bronchiolitis), and for a few endpoints, the seasonal group had more changes (e.g., increased lung collagen content and increased chest wall compliance), suggesting delayed lung maturation. Qualitatively similar changes have been observed in rats exposed to such 'on-off' regimens. Rats also have been exposed to urban patterns of ozone (0.06 ppm background 7 days/week on which were superimposed 9-hour peaks, 5 days/week, slowly rising to and falling from 0.25 ppm) for up to 78 weeks [12].

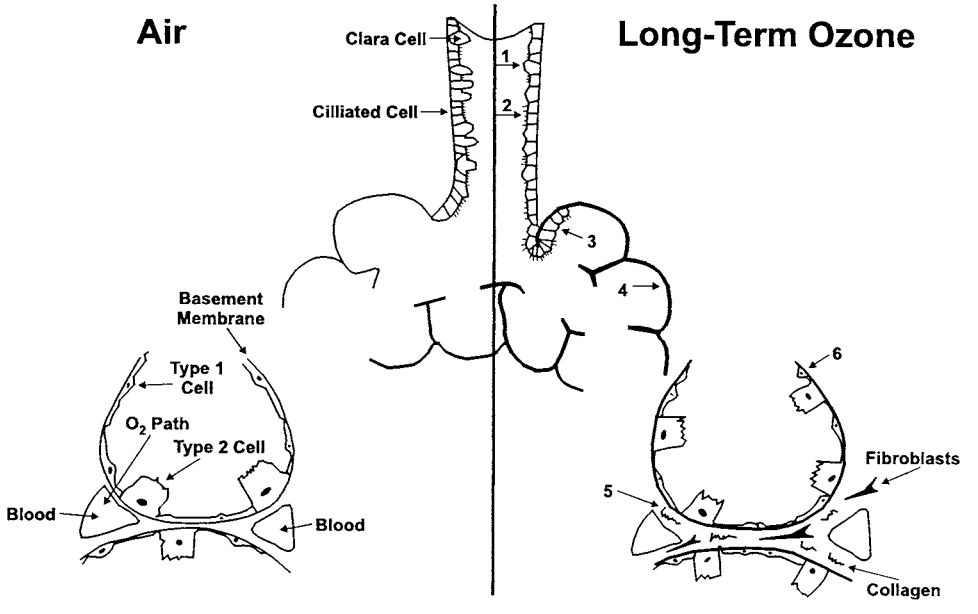


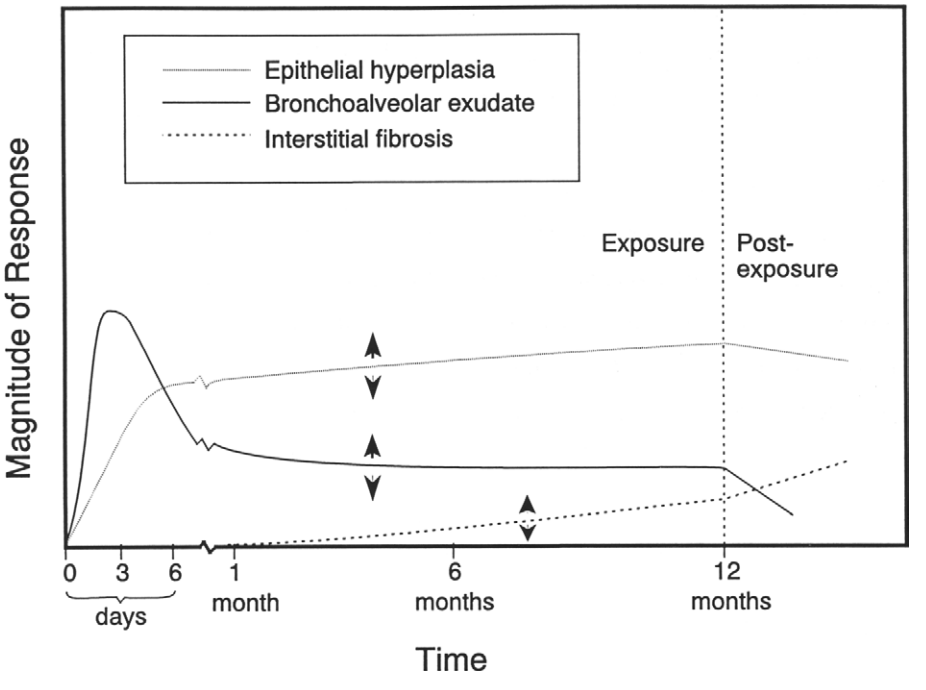
Figure 3. Schematic of zone-induced structural changes in terminal bronchiolus and centriacinar regions of the lung. 1=nonciliated cells which increase and/or have altered shapes, 2=ciliated cells which lose cilia or are sloughed off, 3=bronchiolar epithelium which begins to extend into the alveoli, 4=thickened epithelium, 5=thickened interstitium, 6=thickened basement membrane. Also shown (unnumbered) is an increase in fibroblasts and collagen in the interstitium and a decrease in the number of Type 1 cells accompanied by an increase in the number of Type 2 cells (modified [1]).

Effects observed in the centriacinar region were similar to those described above. Interestingly, many of the changes resolved by 17 weeks after exposure ceased, but the epithelial and endothelial basement membrane were thickened and accompanied by increased collagen fibers at this 17-week postexposure period. These findings had functional correlates indicating that the lung was indeed stiffer perhaps due to the remodelling of the distal airway-alveolar interface [13,14].

3.2. The Quantitative Extrapolation

The impact of ozone on increasing the thickness of the acellular and total interstitial volumes in the centriacinar region was chosen as the effect to be extrapolated because of the importance of this region to the overall function of the lung and the availability of high quality data over a range of concentrations and durations in more than one species of animal. The previous discussion refers to the centriacinar region.

Figure 4. Schematic comparison of the duration-response profiles for the centriacinar region of the lung exposed to a constant low concentrations of ozone [15].



The studies to be used here involved morphometric analyses of a subregion, called the proximal alveolar region (PAR), which for the purposes of this paper can be considered equivalent to the centriacinar region. The general approach taken was to convert the exposure-response information to dose-response curves for these animal studies using dosimetry models and then compare these curves to the doses calculated from exposure models of children and adults. Many assumptions, with varying and sometimes unknown validity, were used. For example, it was assumed that there was a relationship between effect and total cumulative dose to the target tissue. One of the most significant assumptions is that the rate of change of interstitial thickness is related to the rate of ozone uptake. This ignores potential differences in species sensitivity to defensive or reparative mechanisms. Even so, given the likely irreversibility of fibrogenesis and degenerative lung disease, such an assumption is not extreme. More specifics follow (see the ozone criteria document [1], Chapter 8, for details).

The two rat studies selected were reported by Chang and co-workers [12,16]. The initial one used the urban pattern of exposure mentioned above (background of 0.06 ppm on which were superimposed a 9-hour spike of 0.25 ppm, 5 days/week) for up to 78 weeks with periodic measurements; the later study used rats exposed for 5 days/week (6 hours/day) to 0.12, 0.50, or 1.0 ppm for up to 87 weeks. The nonhuman primate studies selected used bonnet monkeys exposed for 90 days (8 hours/day) to 0.15 or 0.3 ppm [17] and cynomolgus monkeys exposed to 0.25 ppm (8 hours/day, 7 days/week), for 18 consecutive months or for every other month for a total of 9 months of exposure over the 18-month period [18].

Figure 5 shows these dose-response curves for the rats and monkeys. The similarity in the dose-response between the two different rat studies and the two different monkey studies is notable, increasing confidence in the representativeness for these species. As can be seen, the monkey is more responsive than the rat. The reason is unknown, but in addition to differences in species sensitivity, it partly may be because of the rats being exposed during the daytime, when they are more quiet (i.e., reduced ventilation and hence ozone dose). The data show an apparent linear dose-response, which is well supported by the strong correlation coefficients. This gives credence to the opinion that interstitial injury is cumulative with exposure.

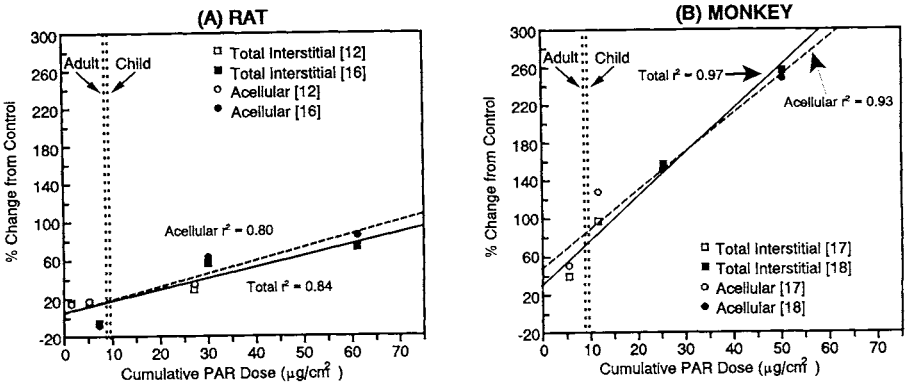


Figure 5. Extrapolation of chronic effects from a laboratory animals to humans. The solid lines represent the linear regression for total interstitial thickness, and the dashed line represents the linear regression for acellular thickness for both studies in each panel of the figure [1].

For the human part of the extrapolation, a hypothetical New York City adult outdoor worker and a 9-year-old New York City child who plays outdoors were selected from the population groups used for the risk assessment prepared by the U.S. Environmental Protection Agency [19]. The activity patterns and ozone exposure information was obtained from the probabilistic NAAQS exposure model [20] using data collected in New York City from April through October 1991. These activity patterns and concentrations were extrapolated to estimate the dose to the PAR of the subjects using a mathematical dosimetry model of Miller and co-workers [21] and Overton and co-workers [21] and assumptions about anatomy, mass transfer coefficients, and ventilation of the selected subjects. The model predicted that the theoretical child would have received a cumulative dose of 9.9 $\mu g/cm^2$ to the PAR over the 214 days of the ozone-season; the adult outdoor worker would have received a cumulative dose of 8.6 $\mu g/cm^2$. Comparing these doses to those on the rat and monkey dose-response curves suggest that a child might experience a 20 to 75% increase in PAR thickness and an adult might have a 15 to 70% increase over the predicted ozone season. The ranges are bounded by the rat curves (low end) and monkey curves (high end). There is no

evidence to determine whether the rat or the monkey better represent the potential human response. However, it is reasonable to assume that the human response lies somewhere in between.

Prolonged exposures cause changes in the distal lung and airways of monkeys and rats that appear consistent with incipient peribronchiolar fibrogenesis within the interstitium and these changes may progress even when exposure ceases. The animal studies have also demonstrated that these structural changes do not reverse between ozone seasons. Thus, the estimate that a child and an adult with a 'real-world' ozone exposure receive a dose that may increase PAR thickness is of concern, especially since only one "season" for the humans was plotted.

4. CONCLUSIONS

The results of short-term animal toxicology, human clinical, and epidemioly studies are strongly correlated. This supports a homology of responses across species, which is strengthened by theoretical equivalencies based on hypothesized mechanisms of action of ozone. Examining the wide array of effects of ozone, the laboratory animals continue to provide more qualitative reasons to be concerned about humans. The quantitative extrapolation by necessity contains numerous assumptions. Nevertheless, it suggests that children and adult workers who spend time outdoors may receive a cumulative dose of ozone during a single season sufficient to increase thickness of the interstitium in the PAR of the lung. In spite of the many uncertainties about the degree of response and its medical interpretation, there is reason for concern that long-term ozone exposure could impart a chronic effect in humans.

REFERENCES

1. U.S. Environmental Protection Agency. (1996) Air quality criteria for ozone and other photochemical oxidants. Research Triangle Park, NC: Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office; EPA report nos. EPA-600/P-93/004aF-cF. 5v. Available from: NTIS, Springfield, VA.
2. Driscoll, K.E.; Vollmuth, T.A.; Schlesinger, R.B. (1987) Acute and subchronic ozone inhalation in the rabbit: response of alveolar macrophages. J. Toxicol. Environ. Health 21: 27-43.
3. Devlin, R.B.; McDonnell, W.F.; Mann, R.; Becker, S.; House, D.E.; Schreinemachers, D.; Koren, H.S. (1991) Exposure of humans to ambient levels of ozone for 6.6 hours causes cellular and biochemical changes in the lung. Am. J. Respir. Cell Mol. Biol. 4: 72-81.
4. Miller, F.J.; Illing, J.W.; Gardner, D.E. (1978) Effect of urban ozone levels on laboratory-induced respiratory infections. Toxicol. Lett. 2: 163-169.
5. Pinkerton, K.E.; Brody, A.R.; Miller, F.J.; Crapo, J.D. (1989) Exposure to low levels of ozone results in enhanced pulmonary retention of inhaled asbestos fibers. Am. Rev. Respir. Dis. 140: 1075-1081.
6. Horstman, D.H.; Folinsbee, L.J.; Ives, P.J.; Abdul-Salaam, S.; McDonnell, W.F. (1990) Ozone concentration and pulmonary response relationships for 6.6-hour exposures with five hours of moderate exercise to 0.08, 0.10, and 0.12 ppm. Am. Rev. Respir. Dis. 142: 1158-1163.
7. Hazucha, M.J. (1987) Relationship between ozone exposure and pulmonary function changes. J. Appl. Physiol. 62: 1671-1680.
8. Costa, D.L.; Stevens, M.S.; Tepper, J.S. (1988) Repeated exposure to ozone (O_3) and chronic lung disease: recent animal data. Presented at: 81st annual meeting of the Air Pollution Control Association; June; Dallas, TX. Pittsburgh, PA: Air Pollution Control Association; paper no. 88-122.3.
9. Hackney, J.D.; Linn, W.S.; Mohler, J.G.; Collier, C.R. (1977) Adaptation to short-term respiratory effects of ozone in men exposed repeatedly. J. Appl. Physiol.: Respir. Environ. Exercise Physiol. 43: 82-85.
10. Tepper, J.S.; Costa, D.L.; Lehmann, J.R.; Weber, M.F.; Hatch, G.E. (1989) Unattenuated structural and biochemical alterations in the rat lung during functional adaptation to ozone. Am. Rev. Respir. Dis. 140: 493-501.
11. Miller, F.J.; Overton, J.H.; Gerrity, T.R.; Graham, R.C. (1988) Interspecies

dosimetry of reactive gases. In: Mohr, U.; Dungworth, D.; McClellan, R.; Kimmerle, G.; Stöber, W.; Lewkowski, J., eds. Inhalation toxicology: the design and interpretation of inhalation studies and their use in risk assessment. New York, NY: Springer-Verlag; pp. 139-155.

12. Chang, L.-Y.; Huang, Y.; Stockstill, B.L.; Graham, J.A.; Grose, E.C.; Ménache, M.G.; Miller, F.J.; Costa, D.L.; Crapo, J.D. (1992) Epithelial injury and interstitial fibrosis in the proximal alveolar regions of rats chronically exposed to a simulated pattern of urban ambient ozone. Toxicol. Appl. Pharmacol. 115: 241-252.
13. Tepper, J.S., Wiester, M.J., Weber, M.F., Fitzgerald, S., and Costa D.L. (1991) Chronic exposure to a simulated urban profile of ozone alters ventilatory responses to CO_2 challenge in rats. Fund. Appl. Toxicol. 17: 52-60.
14. Costa, D.L., Tepper, J.S., Stevens, M.A., Watkinson, W.P., Doerfler, D.L., Gelzleichter, T.R., and Last, J.A. (1995) Restrictive lung disease in rats exposed chronically to an urban profile of ozone. Am. J. Resp. Crit. Care Med. 151: 1512-18.
15. Dungworth, D. L. (1989) Noncarcinogenic responses of the respiratory tract to inhaled toxicants. In: McClellan, R.O.; Henderson, R.F., eds. Concepts in Inhalation Toxicology. New York, NY: pp. 273-298.
16. Chang, L.-Y.; Stockstill, B.L.; Ménache, M.G.; Mercer, R.R.; Crapo, J.D. (1995) Consequences of prolonged inhalation of ozone on F344/N rats: collaborative studies. Part VIII. Morphometric analysis of structural alterations in alveolar regions. Cambridge, MA: Health Effects Institute; pp. 3-39; research report no. 65.
17. Harkema, J.R.; Plopper, C.G.; Hyde, D.M.; St. George, J.A.; Wilson, D.W.; Dungworth, D.L. (1993) Response of macaque bronchiolar epithelium to ambient concentrations of ozone. Am. J. Pathol. 143: 857-866.
18. Tyler, W.S.; Tyler, N.K.; Last, J.A.; Gillespie, M.J.; Barstow, T.J. (1988) Comparison of daily and seasonal exposures of young monkeys to ozone. Toxicology 50: 131-144.
19. U.S. Environmental Protection Agency. (1995) Review of national ambient air quality standards for ozone assessment of scientific and technical information. Research Triangle Park, NC: Office of Air Quality Planning and Standards; OAQPS staff paper.
20. Johnson, T.R. (1994) One-minute ozone exposure sequences for selected New York cohorts [letter to Dr. John H. Overton, U.S. EPA]. Durham, NC: International Technology Corporation; November 22.

21. Miller, F.J.; Overton, J.H., Jr.; Jaskot, R.H.; Menzel, D.B. (1985) A model of the regional uptake of gaseous pollutants in the lung: I. the sensitivity of the uptake of ozone in the human lung to lower respiratory tract secretions and exercise. Toxicol. Appl. Pharmacol. 79: 11-27.

22. Overton, J.H., Jr.; Graham, R.C.; Miller, F.J. (1987) A model of the regional uptake of gaseous pollutants in the lung: II. the sensitivity of ozone uptake in laboratory animal lungs to anatomical and ventilatory parameters. Toxicol. Appl. Pharmacol. 88: 418-432.